

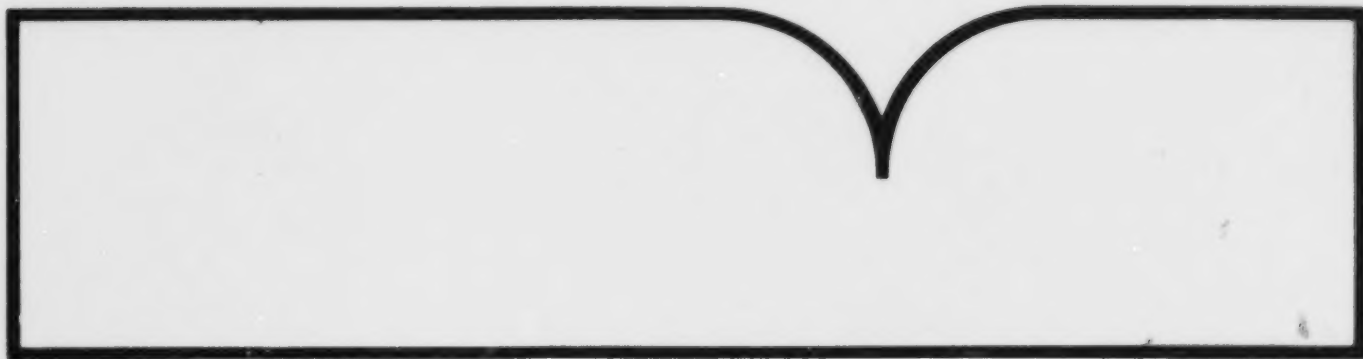
Environmental Assessment of a Low-Emission
Oil-Fired Residential Hot Water Condensing
Heating System. Volume II: Data Supplement

Acurex Corp.
Mountain View, CA

Prepared for

Industrial Environmental Research Lab.
Research Triangle Park, NC

May 82



U.S. Department of Commerce
National Technical Information Service

NTIS



Research and Development

ENVIRONMENTAL ASSESSMENT OF
A LOW-EMISSION OIL-FIRED
RESIDENTIAL HOT WATER
CONDENSING HEATING SYSTEM
Volume II. Data Supplement

Prepared for

Office of Air Quality Planning and Standards

Prepared by

Industrial Environmental Research
Laboratory
Research Triangle Park NC 27711

TECHNICAL REPORT DATA (Please read Instructions on the reverse before completing)			
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15. SUPPLEMENTARY NOTES IERL-RTP project officer is Robert E. Hall, Mail Drop 65, 919/541-2477. Volume I. gives technical results.			
16. ABSTRACT The report gives results of a test program measuring air and water emissions from a high-efficiency hot-water residential heating system of European design, utilizing a condensing flue gas system and a low emission burner. Criteria and noncriteria emissions, including trace elements and organic species in both flue gas and condensate waste water streams, were measured. NO (as NO ₂), CO, total UHC (as propane), and total particulate emissions measured about 37, 12, 1.5, and 2.7 ng/J heat input, respectively. Absorption of sulfates and nitrates in the waste water resulted in a constant pH of 3.0. Total organic emissions in the flue gas measured 3.5 mg/dscm; they were below the detectable limit in the waste water. Several inorganic trace elements, including chromium, copper, iron, and nickel, in the waste water were attributed to leaching of heat transfer metal surfaces by the warm acidic water. Bioassays were also performed to evaluate the potential health hazard of the streams. Results indicate nondetectable to moderate toxicity and mutagenicity.			
17. KEY WORDS AND DOCUMENT ANALYSIS			
a. DESCRIPTORS		b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Pollution Waste Water		Pollution Control	13B
Assessments Condensates		Stationary Sources	14B 07D
Hot Water Heating Measurement		Environmental Assessment	13A
Residential Buildings			13M
Fuel Oil			21D
Flue Gases			21B
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ENVIRONMENTAL ASSESSMENT OF A LOW EMISSION OIL-FIRED RESIDENTIAL
HOT WATER CONDENSING HEATING SYSTEM

VOLUME II: DATA SUPPLEMENT

April 1982

Acurex Project 7600

Contract 68-02-3188

For

EPA Project Officer - R. E. Hall
Combustion Research Branch
Energy Assessment and Control Division
Industrial Environmental Research Laboratory
Research Triangle Park, North Carolina 27711

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NOTICE

**Mention of trade names or commercial products does not
constitute endorsement or recommendation for use.**

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SECTION 1
INTRODUCTION

The purpose of this Data Supplement is to document data in greater detail than was practical in the final report (reference 1-1). It is intended to provide sufficient detail for researchers to perform their own analysis of the data. Readers are referred to the final report for objectives, description of the emission results, interpretations, and conclusions.

The data supplement contains the following information:

- Preliminary equipment calibration -- calibration data for the dry gas meters used to sample flue gas for both EPA Method 5 and Source Assessment Sampling System (SASS) trains
- Complete furnace operating data -- furnace water and flue gas temperatures throughout each burner-on/burner-off cycle; cooling water flowrate, and thermostat settings
- Sampling data sheets -- emission data obtained with continuous monitoring instrumentation: operating data tables for EPA Method 5 (for particulate mass emissions), EPA Method 8 (for SO₂ and SO₃ sampling), and SASS (for particulate, trace element, and organic sampling)
- Analytical laboratory results -- ultimate analysis of distillate fuel oil used in the test program: laboratory

analysis reports on particulate levels by gravimetric analysis; sulfur by turbimetric analysis; trace elements by Spark Source Mass Spectrometry (SSMS) analysis and Atomic Absorption (AA) analysis; anions by ion chromatography; Total Chromatographable Organic (TCO) emissions by onsite Gas Chromatography (GC); speciation of organic emissions by Gas Chromatography/Mass Spectrometry (GC/MS); biological assays of the extract and water discharge samples

- SAM IA work sheets -- Source Assessment Model IA evaluation of emission and effluent data to determine the potential hazard posed by both flue gas and water discharge samples

REFERENCE FOR SECTION 1

- 1-1. Castaldini, C., "Environmental Assessment of a Low-NO_x Residential Hot Water Condensing Heating System Burning Distillate Oil, Volume 1: Technical Results."

SECTION 2
PRELIMINARY EQUIPMENT CALIBRATION

Date 5-7-80
 Time 1341
 Barometric Pressure 30.16
 Ambient Temperature 70°

Orifice Meter
 Orifice Magnehelic 702219M28
 Primary Calibration Meter 433583
 Control Module #043

Operators BRANAM
 Wet Bulb Temperature

METER CALIBRATION DATA

Orifice Manometer ΔH_{ref} (in. wg.)	Orifice Magnehelic ΔH_0 (in. wg.)	Primary Meter ΔH_p (in. wg.)	Dry Test Meter P_{dg} (in. wg.)	Gas Volume Primary Meter V_p (ft. ³)	Gas Volume Dry Test Meter V_d (ft. ³)	Temperature						Time t (min.)	α	K_0	
						Primary Meter			Dry Test Meter						
						Inlet, T_{pi} (°F)	Outlet, T_{po} (°F)	Avg., T_{pa} (°F)	Inlet, T_{di} (°F)	Outlet, T_{do} (°F)	Avg., T_{da} (°F)				
	0.2														
	0.4														
	0.6														
	0.8			663.147	962.379	74	76	78	72	72	72.0	6.0	494	3685	
				679.420	978.339	86	76		72	72					
	1.0			16.273	15.96										
	1.2														
	1.4														
	1.6														
	1.8			679.420	978.329	80	76		72	72					
				699.999	977.547	97	78	82.75	73	71	72.0	5.0	955	3431	
	2.0			20.579	19.208										
	2.2														
	2.4														
	2.6														
	2.8			699.999	997.548	87	79		73	72					
				718.174	016.430	102	81	86.0	74	72	72.75	4.0	1072	3477	
	3.0			18.175	18.882										
	3.2														
	3.4														
	3.6														
	3.8			718.174	016.430	91	82		73	73					
				734.576	038.699	101	84	89.5	73	75	73.5	3.0	1009	3774	
	4.0			16.402	16.269										
	4.2														
	4.4														
	4.6														
	4.8														
	5.0														
Average														1.063	3.722

Date 5-7-80
Time 1200
Barometric Pressure 30.16
Ambient Temperature 70°

Orifice Meter
Orifice Magnehelic 70219 m28
Primary Calibration Meter 433583
Control Module # 043

Operators BEAUM
Wet Bulb Temperature

METER CALIBRATION DATA

Driflce Manometer ΔH_{ref} (in.wg.)	Driflce Magnehelic ΔH_0 (in.wg.)	Primary Meter ΔH_p (in.wg.)	Dry Test Meter P_{dg} (in. wg.)	Gas Volume Primary Meter V_p (ft.³)	Gas Volume Dry Test Meter V_d (ft.³)	Temperature						Time t (min.)	α	K ₀	
						Primary Meter			Dry Test Meter						
						Inlet, T_{pi} (°F)	Outlet, T_{po} (°F)	Avg., T_{pa} (°F)	Inlet, T_{di} (°F)	Outlet, T_{do} (°F)	Avg., T_{da} (°F)				
	0.2														
	0.4														
	0.6														
	0.8			594.163 614.863	900.115 915.426	66 68	66 68	67.0	68 71	70 71	70.0	31.0	1.028	.677	
	1.0			15.1	15.311										
	1.2														
	1.4														
	1.6														
	1.8			614.863 630.899	915.426 931.049	70 72	70 72	71.0	68 69	69 69	68.5	21.0	.964	.731	
	2.0			16.036	15.623										
	2.2														
	2.4														
	2.6														
	2.8			630.899 646.840	931.049 946.554	72 74	72 74	73.5	70 70	70 70	70.0	17.0	.447	.719	
	3.0			15.941	15.505										
	3.2														
	3.4														
	3.6														
	3.8			646.840 663.148	946.554 962.379	73 74	73 74	75.5	70 70	70 70	70.0	15.0	.441	.716	
	4.0			16.308	15.825										
	4.2														
	4.4														
	4.6														
	4.8														
	5.0														
Average															
														.493	.716

1-21391

Date 5-7-80
 Time 921
 Barometric Pressure 30.02
 Ambient Temperature 70°

Orifice Meter
 Orifice Magnehelic 5062-3AN54
 Primary Calibration Meter 433583
 Control Module CR1

Operators BEANAM
 Wet Bulb Temperature

METER CALIBRATION DATA

Orifice Manometer ΔH_{ref} (in. wg.)	Orifice Magnehelic ΔH_0 (in. wg.)	Primary Meter ΔH_p (in. wg.)	Dry Test Meter P_{dg} (in. wg.)	Gas Volume Primary Meter V_p (ft. ³)	Gas Volume Dry Test Meter V_d (ft. ³)	Temperature						Time t (min.)	α	K_0	
						Primary Meter			Dry Test Meter						
						Inlet, T_{pi} (°F)	Outlet, T_{po} (°F)	Avg., T_{pa} (°F)	Inlet, T_{di} (°F)	Outlet, T_{do} (°F)	Avg., T_{da} (°F)				
	0.2														
	0.4														
	0.6														
	0.8			531.811 547.623	184.100 194.711	65 65	65	65.0	66 74	65 66	67.75	6.0			
	1.0			15.812	15.611										
	1.2														
	1.4														
	1.6														
	1.8			547.623 563.126	194.711 215.065	65 66	65	65.25	70 74	66 68	69.5	4.0			
	2.0			15.503	15.354										
	2.2														
	2.4														
	2.6														
	2.8			563.126 581.891	215.065 233.770	65 66	65	65.5	72 78	68 70	72	4.0			
	3.0			18.765	18.705										
	3.2														
	3.4														
	3.6														
	3.8			581.891 597.713	233.770 249.665	65 65	65	65.0	76 78	66 69	70	3.0	1003		
	4.0			15.822	15.895										
	4.2														
	4.4														
	4.6														
	4.8														
	5.0														
Average															

SECTION 3
COMPLETE FURNACE OPERATING DATA

FURNACE OPERATING DATA

* EACH ENTRY REPRESENTS ONE MINUTE INTERVAL

Cycle No.	Cooling Water Flowrate (gpm)	Thermostat Setting (°C)	Inlet Water Temperature °F	Outlet Water Temperature °F	Tank Water Temperature °F	Exhaust Duct Temperature °F	Ambient Temperature °F
1	1.7	53	56	68	97	61	76
			56	71	101	63	77
			56	76	107	68	74
			56	80	113	73	75
			56	84	119	76	74
			56	87	124	78	77
			56	90	129	79	77
			56	92	133	80	77
			56	94	136	82	80
			56	97	140	82	79
			56	99	144	83	80
			56	100	147	83	80
			56	103	151	83	77
			56	103	154	83	90
			56	100	153	81	99
			56	95	149	80	98
			56	93	145	78	96
			56	90	141	77	97
			56	88	138	77	97
			56	87	134	77	93
			56	84	132	76	93
			56	83	129	75	93
	✓	✓	56	81	126	74	92

FURNACE OPERATING DATA

Cycle No.	Cooling Water Flowrate (gpm)	Thermostat Setting (°C)	Inlet Water Temperature (°F)	Outlet Water Temperature (°F)	Tank Water Temperature (°F)	Exhaust Duct Temperature (°F)	Ambient Temperature (°F)
2	1.7	53	56	80	124	74	92
			56	79	121	72	90
			56	78	119	72	90
			56	77	117	72	89
			56	75	115	72	89
			56	74	113	72	88
			56	74	111	72	87
			56	73	109	72	87
			56	72	107	72	87
			56	72	106	73	83
			56	71	104	72	85
			56	70	103	73	83
			56	70	101	73	82
			56	69	100	73	84
			56	68	98	73	85
			56	68	97	61	76
		53	56	71	100	63	73
	1.7		56	77	106	68	71
			56	81	112	73	75
			56	84	118	76	77
			56	87	123	78	75
			56	90	128	79	74
			56	92	132	80	74

FURNACE OPERATING DATA

3

Cycle No.	Cooling Water Flowrate (gpm)	Thermostat Setting (°C)	Inlet Water Temperature °F	Outlet Water Temperature °F	Tank Water Temperature °F	Exhaust Duct Temperature °F	Ambient Temperature °F
	1.7	53	56	94	136	81	72
			56	97	140	81	73
			56	98	144	83	69
			56	100	147	83	72
			55	102	151	83	72
			55	104	153	84	72
			55	105	156	85	71
			55	102	156	79	82
			55	99	153	77	80
			55	94	149	73	75
			54	90	145	72	78
			55	88	141	72	74
			55	87	138	71	72
			55	86	135	70	79
			55	83	132	69	73
			54	81	129	68	72
			54	80	126	68	70
			55	78	123	68	69
			54	78	121	67	69
			54	77	119	67	69
			55	76	117	67	69
			54	75	114	67	69
			55	73	112	67	68

FURNACE OPERATING DATA

Cycle No.	Cooling Water Flowrate (gpm)	Thermostat Setting (°C)	Inlet Water Temperature °F	Outlet Water Temperature °F	Tank Water Temperature °F	Exhaust Duct Temperature °F	Ambient Temperature °F
3	1.7	53	55	72	110	67	69
			55	71	108	67	67
			55	71	107	66	65
			55	70	105	59	65
	1.7	53.5	54	73	107	62	64
			55	78	113	68	65
			55	83	118	73	66
			55	87	123	77	65
			55	89	128	79	67
			55	91	133	80	65
4			55	93	137	81	68
			54	96	141	81	69
			55	98	145	82	68
			55	101	148	83	67
			55	102	151	84	71
			55	104	154	85	68
			55	106	157	86	69
			55	108	160	86	69
			55	109	162	87	75
			56	105	162	83	83
5			55	100	157	81	85
			55	97	153	78	84
			55	94	149	76	84
			55	94	149	76	84

FURNACE OPERATING DATA

Cycle No.	Cooling Water Flowrate (gpm)	Thermostat Setting (°C)	Inlet Water Temperature °F	Outlet Water Temperature °F	Tank Water Temperature °F	Exhaust Duct Temperature °F	Ambient Temperature °F
4	1.7	53.5	55	91	146	74	83
			55	90	142	72	86
			56	88	138	72	79
			55	85	135	70	79
			55	84	132	69	75
			55	81	129	68	76
			55	80	126	68	75
			54	80	124	67	77
			55	77	121	67	76
			55	77	119	66	74
			55	76	117	66	77
			55	74	114	67	77
			55	74	113	67	75
			55	73	111	66	72
			55	72	109	66	70
			55	72	107	66	72
			55	71	106	66	73
			55	70	104	61	68
	1.7	53.5	55	74	107	64	67
			55	74	113	70	68
			55	83	117	74	68
			55	87	123	77	70
			55	89	128	79	69

FURNACE OPERATING DATA

6

Cycle No.	Cooling Water Flowrate (gpm)	Thermostat Setting (°C)	Inlet Water Temperature °F	Outlet Water Temperature °F	Tank Water Temperature °F	Exhaust Duct Temperature °F	Ambient Temperature °F
	1.7	53.5	55	93	132	80	70
			55	94	136	80	71
			55	96	140	82	68
			55	99	145	83	70
			56	100	147	83	72
			56	102	150	84	72
			55	104	153	85	72
			55	106	156	86	71
			55	107	159	87	73
			56	107	160	83	84
			55	101	157	81	88
			56	97	153	79	83
			55	94	149	77	84
			56	92	145	74	80
			55	90	142	73	84
			56	88	138	73	86
			56	86	135	71	86
			55	84	132	71	83
			55	83	129	71	76
			56	82	127	71	80
			56	80	124	71	82
			56	80	121	71	80
	✓	✓	56	79	119	70	79

FURNACE OPERATING DATA

7

Cycle No.	Cooling Water Flowrate (gpm)	Thermostat Setting (°C)	Inlet Water Temperature °F	Outlet Water Temperature °F	Tank Water Temperature °F	Exhaust Duct Temperature °F	Ambient Temperature °F
5	1.7	53.5	55	77	117	70	78
			56	76	115	70	77
			55	76	113	70	77
			56	74	111	70	79
			55	73	109	70	75
			56	72	107	71	77
			56	72	106	72	74
			56	72	104	71	74
			56	71	103	62	71
			56	75	107	65	70
	1.7	53.5	56	80	112	71	72
			56	84	117	74	70
			56	87	122	77	70
			56	90	127	74	71
			56	92	132	80	73
			56	94	136	81	75
			56	97	139	82	72
			56	99	143	83	72
			56	100	147	83	74
			56	103	150	85	74
			56	104	153	85	74
			56	107	156	86	77
			56	109	158	86	81

FURNACE OPERATING DATA

8

Cycle No.	Cooling Water Flowrate (gpm)	Thermostat Setting (°C)	Inlet Water Temperature °F	Outlet Water Temperature °F	Tank Water Temperature °F	Exhaust Duct Temperature °F	Ambient Temperature °F
	1.7	53.5	56	104	158	82	87
			56	99	154	79	90
			56	96	150	77	91
			56	93	146	75	92
			56	91	142	74	85
			56	89	139	74	90
			56	87	135	74	89
			56	85	133	73	86
			56	84	130	73	84
			56	83	127	72	84
			56	81	125	71	81
			56	80	122	70	84
			56	79	119	71	81
			56	78	117	69	82
			56	77	115	69	80
			56	76	114	71	82
			56	75	111	72	79
			56	74	110	72	79
			56	73	108	72	77
			56	73	106	71	77
			56	72	104	71	77
			56	72	103	71	78
✓		✓	56	71	102	62	73

FURNACE OPERATING DATA

Cycle No.	Cooling Water Flowrate (gpm)	Thermostat Setting (°C)	Inlet Water Temperature °F	Outlet Water Temperature °F	Tank Water Temperature °F	Exhaust Duct Temperature °F	Ambient Temperature °F
6	1.7	53.5	56	73	103	63	73
			56	79	108	68	73
			56	81	113	72	72
			56	85	118	77	73
			56	88	124	78	73
			56	91	128	80	72
			56	93	133	80	74
			56	96	137	82	75
			56	97	141	82	75
			56	100	145	83	77
			56	102	148	83	77
			56	104	151	84	77
			56	105	154	84	75
			56	107	157	85	80
			56	108	156	91	84
			56	94	153	79	90
			56	94	148	77	92
			56	92	145	76	88
			56	90	141	75	79
			56	88	138	74	83
			56	86	134	73	89
			56	84	132	74	88
			56	83	129	74	87

FURNACE OPERATING DATA

10

Cycle No.	Cooling Water Flowrate (gpm)	Thermostat Setting (°C)	Inlet Water Temperature (°F)	Outlet Water Temperature (°F)	Tank Water Temperature (°F)	Exhaust Duct Temperature (°F)	Ambient Temperature (°F)
	1.7	53.5	56	82	126	73	80
			56	80	124	74	80
			56	80	121	74	82
			56	79	118	74	79
			56	77	117	73	83
			56	77	114	73	80
			56	76	113	73	83
			56	75	110	72	80
			56	74	109	72	80
			56	73	107	72	81
			56	73	106	72	80
			56	72	104	72	81
			56	72	102	73	82
			56	71	101	63	78
		↓	56	72	101	63	77
7	1.7	53.5	57	77	106	68	78
			56	82	111	73	75
			56	86	117	77	73
			57	87	122	79	75
			57	90	127	80	74
			56	93	131	81	75
			56	95	135	82	75
	↓	↓	56	94	140	83	73

FURNACE OPERATING DATA

11

Cycle No.	Cooling Water Flowrate (gpm)	Thermostat Setting (°C)	Inlet Water Temperature °F	Outlet Water Temperature °F	Tank Water Temperature °F	Exhaust Duct Temperature °F	Ambient Temperature °F
	1.7	53.5	56	89	144	83	75
			56	101	146	83	76
			57	103	150	84	76
			57	105	153	85	77
			56	107	155	85	83
			56	103	155	83	89
			56	99	152	81	94
			57	96	148	81	95
			57	93	145	80	93
			57	90	141	80	90
			57	89	137	78	90
			56	87	134	77	92
			56	86	131	75	94
			56	84	129	73	91
			57	83	125	74	89
			56	82	124	73	87
			56	81	121	74	89
			57	80	119	73	91
			56	78	116	73	88
			56	77	114	74	85
			56	77	113	73	84
			56	76	110	72	87
	✓	✓	56	75	109	72	83

FURNACE OPERATING DATA

Cycle No.	Cooling Water Flowrate (gpm)	Thermostat Setting (°C)	Inlet Water Temperature °F	Outlet Water Temperature °F	Tank Water Temperature °F	Exhaust Duct Temperature °F	Ambient Temperature °F
	1.7	53.5	57	74	101	72	84
			56	73	105	74	85
			56	72	104	75	83
			57	72	102	75	79
			56	72	101	74	81
			56	71	100	73	80
		↓	56	70	98	63	77
	1.7	53.5	56	72	100	64	76
			56	71	106	64	80
			56	81	111	73	79
			56	84	117	77	78
			56	87	122	79	77
			57	90	126	80	78
			57	93	131	80	79
			57	95	135	81	78
			56	97	139	82	79
			56	99	143	82	77
			56	101	146	82	78
			56	103	147	83	80
			56	105	152	83	80
			56	103	154	83	91
			56	94	151	91	92
	↓	↓	57	94	147	80	86

FURNACE OPERATING DATA

13

Cycle No.	Cooling Water Flowrate (gpm)	Thermostat Setting (°C)	Inlet Water Temperature °F	Outlet Water Temperature °F	Tank Water Temperature °F	Exhaust Duct Temperature °F	Ambient Temperature °F
	1.7	53.5	56	92	143	78	91
			56	90	139	77	96
			57	88	136	77	90
			56	87	133	77	94
			57	85	131	75	88
			56	84	128	76	87
			57	83	125	77	87
			56	81	123	76	77
			57	80	120	74	93
			56	79	118	73	85
			56	77	116	72	86
			56	77	114	74	88
			57	76	112	73	88
			57	76	110	72	83
			57	75	109	73	83
			57	74	107	75	86
			57	73	105	74	77
			57	72	104	73	79
			56	72	103	72	81
			57	71	101	73	81
			56	70	100	67	80
	↓	✓	57	71	99	63	80
9	1.7	55	57	75	103	66	82

FURNACE OPERATING DATA

14

Cycle No.	Cooling Water Flowrate (gpm)	Thermostat Setting (°C)	Inlet Water Temperature °F	Outlet Water Temperature °F	Tank Water Temperature °F	Exhaust Duct Temperature °F	Ambient Temperature °F
	1.7	55	56	80	108	71	80
			57	83	114	76	80
			56	87	119	77	82
			56	89	124	79	79
			56	92	129	80	80
			57	94	133	81	79
			56	96	137	81	79
			56	97	141	81	78
			57	100	144	82	79
			56	103	148	83	81
			57	105	150	84	84
			57	101	151	82	87
			57	97	148	81	93
			56	94	145	80	90
			57	91	140	79	91
			57	90	137	78	90
			57	88	134	77	93
			57	87	131	77	93
			57	85	129	77	85
			57	83	126	77	90
			56	81	123	77	83
			56	80	121	75	80
	✓	✓	56	79	119	75	79

FURNACE OPERATING DATA

15

Cycle No.	Cooling Water Flowrate (gpm)	Thermostat Setting (°C)	Inlet Water Temperature °F	Outlet Water Temperature °F	Tank Water Temperature °F	Exhaust Duct Temperature °F	Ambient Temperature °F
	1.7	55	56	79	117	74	85
			56	77	114	75	82
			57	76	112	74	85
			57	76	110	74	85
			57	75	109	74	88
			57	74	107	76	77
			56	73	106	76	77
			56	72	104	76	78
			56	72	102	74	78
			56	71	101	74	78
			56	71	100	75	82
	✓	✓	57	70	99	63	77
10	1.7	55	56	72	100	64	75
			56	77	106	69	77
			57	82	112	73	77
			57	85	117	76	78
			56	88	122	77	78
			56	90	126	79	78
			57	92	131	80	78
			56	95	134	81	77
			57	97	138	81	77
			56	99	142	81	78
	✓	✓	56	101	145	82	80

FURNACE OPERATING DATA

16

Cycle No.	Cooling Water Flowrate (gpm)	Thermostat Setting (°C)	Inlet Water Temperature (°F)	Outlet Water Temperature (°F)	Tank Water Temperature (°F)	Exhaust Duct Temperature (°F)	Ambient Temperature (°F)
	1.7	55	56	103	148	83	80
			56	104	152	84	78
			56	105	154	82	84
			56	100	153	80	82
			56	96	149	79	84
			56	93	144	79	89
			56	90	141	78	83
			56	90	141	78	84
			56	90	141	78	82
			56	90	140	77	82
			56	90	140	77	82
			56	90	138	77	83
			56	97	135	77	82
			56	86	132	76	80
			56	83	130	75	77
			55	82	127	75	77
			56				
			56	79			
			56	78			
			56	77	115	73	77
			56	77	114	72	76
4		4	56	76	113	72	77

FURNACE OPERATING DATA

17

Cycle No.	Cooling Water Flowrate (gpm)	Thermostat Setting (°C)	Inlet Water Temperature °F	Outlet Water Temperature °F	Tank Water Temperature °F	Exhaust Duct Temperature °F	Ambient Temperature °F
	1.7	55	56	75	110	72	77
			56	74	109	72	75
			56	73	107	73	74
			56	72	105	72	74
			56	72	104	72	73
			56	71	103	73	76
			56	70	101	62	74
			56	74	105	64	74
			56	79	110	68	75
			56	83	114	72	74
			56	86	120	75	75
			56	89	124	76	76
			56	90	128	77	77
			56	93	133	78	75
			56	95	137	79	77
			56	98	141	80	76
			56	100	145	81	77
			56	102	148	81	79
			57	104	150	82	78
			56	105	153	83	80
			56	104	155	81	81
			56	99	153	80	81
			56	95	149	78	83

FURNACE OPERATING DATA

18

Cycle No.	Cooling Water Flowrate (gpm)	Thermostat Setting (°C)	Inlet Water Temperature °F	Outlet Water Temperature °F	Tank Water Temperature °F	Exhaust Duct Temperature °F	Ambient Temperature °F
	1.7	55	56	93	145	77	80
			56	90	141	77	82
			56	89	138	77	87
			56	87	135	76	81
			56	85	132	75	80
			56	84	129	75	80
			56	83	127	75	80
			56	81	124	75	78
			56	80	121	74	78
			56	79	119	75	83
			56	78	117	75	83
			56	77	115	75	85
			56	77	113	76	84
			56	75	111	77	83
			56	74	109	77	86
			56	74	108	77	86
			56	74	107	78	87
			57	73	105	79	84
			56	72	103	76	85
			56	72	101	74	84
			57	71	100	74	87
			57	71	99	72	84
	✓	✓	57	71	98	63	83

FURNACE OPERATING DATA

19

Cycle No.	Cooling Water Flowrate (gpm)	Thermostat Setting (°C)	Inlet Water Temperature °F	Outlet Water Temperature °F	Tank Water Temperature °F	Exhaust Duct Temperature °F	Ambient Temperature °F
12	1.7	55	57	74	101	67	80
			56	80	107	71	82
			56	83	112	74	82
			57	87	117	77	84
			57	89	122	78	83
			57	91	127	79	84
			56	93	131	79	86
			57	95	134	80	86
			57	97	138	80	87
			57	100	142	81	84
			57	99	145	82	91
			57	94	143	81	95
			57	91	140	79	91
			57	89	137	80	92
			57	87	133	79	94
			57	86	130	79	93
			56	84	128	80	90
			57	83	124	81	91
			56	81	122	78	93
			57	80	120	77	91
			57	79	118	80	90
			57	78	116	80	90
	0	5	57	77	114	80	91

FURNACE OPERATING DATA

20

Cycle No.	Cooling Water Flowrate (gpm)	Thermostat Setting (°C)	Inlet Water Temperature °F	Outlet Water Temperature °F	Tank Water Temperature °F	Exhaust Duct Temperature °F	Ambient Temperature °F
	1.7	55	57	76	112	80	90
			56	76	110	77	87
			57	75	108	80	89
			57	74	107	78	86
			57	74	105	80	86
			57	72	104	78	87
			57	72	102	80	85
			57	72	100	79	85
			57	71	100	80	85
			57	71	98	77	83
			57	70	97	79	84
			57	70	96	77	83
			56	69	95	79	86
			57	69	93	69	83
		↓	57	69	93	64	82
	1.7	55	57	74	98	67	80
			57	78	104	70	83
			56	81	110	73	82
			57	85	115	76	90
			57	87	119	77	80
			57	89	124	77	80
			57	91	129	79	93
	↓	↓	57	93	133	79	81

13

FURNACE OPERATING DATA

21

Cycle No.	Cooling Water Flowrate (gpm)	Thermostat Setting (°C)	Inlet Water Temperature °F	Outlet Water Temperature °F	Tank Water Temperature °F	Exhaust Duct Temperature °F	Ambient Temperature °F
	1.7	55	56	96	136	80	81
			56	98	140	80	83
			57	100	144	81	83
			56	102	147	82	87
			56	102	149	83	100
			56	97	148	83	104
			57	93	144	83	102
			57	91	140	81	99
			56	89	137	82	100
			56	87	134	82	100
			56	85	131	82	98
			57	83	128	81	89
			57	82	125	81	88
			56	81	123	81	89
			57	80	121	81	92
			56	79	119	80	87
			57	77	116	79	86
			56	77	114	77	86
			57	76	112	74	86
			57	75	110	75	87
			57	75	109	78	85
			56	73	107	78	84
	✓	✓	56	73	105	79	94

FURNACE OPERATING DATA

22

Cycle No.	Cooling Water Flowrate (gpm)	Thermostat Setting (°C)	Inlet Water Temperature (°F)	Outlet Water Temperature (°F)	Tank Water Temperature (°F)	Exhaust Duct Temperature (°F)	Ambient Temperature (°F)
	1.7	55	56	72	104	76	86
			57	71	102	78	86
			57	71	101	80	86
			56	70	100	80	87
			56	70	98	80	86
			56	69	97	80	87
			56	69	96	80	87
		U	56	68	95	65	82
			57	71	97	67	81
14	1.7	55	56	76	103	70	81
			57	80	109	73	81
			57	83	113	76	80
			56	86	119	77	81
			57	87	124	78	83
			56	90	128	79	84
			56	92	132	80	84
			56	94	135	80	83
			57	96	139	81	84
			56	99	143	82	85
			57	101	147	82	83
			57	102	149	83	93
			57	97	148	83	94
	U	V	57	94	144	83	93

FURNACE OPERATING DATA

23

Cycle No.	Cooling Water Flowrate (gpm)	Thermostat Setting (°C)	Inlet Water Temperature (°F)	Outlet Water Temperature (°F)	Tank Water Temperature (°F)	Exhaust Duct Temperature (°F)	Ambient Temperature (°F)
	1.7	55	57	91	141	81	89
			57	88	137	81	90
			56	87	134	80	86
			56	85	131	80	89
			56	83	128	78	87
			57	83	126	78	92
			56	81	123	79	89
			57	80	121	77	86
			56	79	118	77	86
			56	78	116	76	87
			57	77	114	76	87
			57	76	112	76	85
			57	75	110	76	87
			57	75	109	77	86
			57	73	107	80	87
			57	73	106	81	87
			57	72	104	81	88
			56	72	103	82	88
			57	71	101	80	87
			56	71	100	80	85
			57	70	98	81	85
	1	J	57	69	97	71	81
15	1.7	55	56	71	99	69	80

FURNACE OPERATING DATA

24

Cycle No.	Cooling Water Flowrate (gpm)	Thermostat Setting (°C)	Inlet Water Temperature °F	Outlet Water Temperature °F	Tank Water Temperature °F	Exhaust Duct Temperature °F	Ambient Temperature °F
	1.7	55	56	76	104	72	81
			56	80	104	74	81
			57	84	114	77	82
			57	87	120	78	81
			56	88	124	78	82
			56	91	128	79	82
			56	93	133	80	82
			57	95	136	81	81
			57	97	140	82	83
			57	99	144	82	82
			56	100	146	82	82
			56	100	149	83	80
			56	96	147	83	98
			56	93	144	82	94
			57	90	141	81	93
			56	88	137	80	92
			57	87	133	82	93
			57	84	131	79	93
			56	83	128	79	90
			56	82	125	79	91
			57	81	123	80	91
			57	80	121	79	87
	V	V	56	78	118	90	86

FURNACE OPERATING DATA

25

Cycle No.	Cooling Water Flowrate (gpm)	Thermostat Setting (°C)	Inlet Water Temperature °F	Outlet Water Temperature °F	Tank Water Temperature °F	Exhaust Duct Temperature °F	Ambient Temperature °F
	1.7	55	56	79	116	80	91
			56	77	114	80	91
			57	76	112	80	89
			57	75	110	81	89
			56	75	108	82	87
			56	74	107	82	90
			56	73	105	81	91
			56	72	104	82	88
			57	72	103	82	87
			57	71	101	81	90
		↓	57	71	100	71	83
		55	57	73	102	71	81
	1.7		57	78	107	73	81
			57	83	112	76	81
			56	85	117	78	83
			56	88	122	79	83
			56	90	123	80	83
			56	93	131	80	83
			56	94	135	80	83
			57	96	139	81	84
			57	98	142	82	84
			57	100	145	82	85
	↓	↓	56	103	144	83	84

16

FURNACE OPERATING DATA

26

Cycle No.	Cooling Water Flowrate (gpm)	Thermostat Setting (°C)	Inlet Water Temperature (°F)	Outlet Water Temperature (°F)	Tank Water Temperature (°F)	Exhaust Duct Temperature (°F)	Ambient Temperature (°F)
	1.7	55	56	101	151	83	95
			57	97	148	83	91
			57	93	146	81	92
			56	91	141	81	89
			57	89	138	81	90
			56	87	135	80	87
			56	86	132	79	87
			56	84	128	80	87
			57	83	127	80	85
			56	82	124	80	89
			57	80	121	80	87
			57	80	119	81	86
			57	78	117	82	88
			57	77	115	82	87
			56	77	113	82	87
			56	76	111	83	86
			57	75	109	82	85
			56	74	107	82	88
			56	73	106	82	87
			56	72	104	83	87
			56	72	103	82	86
			57	71	102	82	87
	✓	✓	57	71	100	82	86

FURNACE OPERATING DATA

21

Cycle No.	Cooling Water Flowrate (gpm)	Thermostat Setting (°C)	Inlet Water Temperature °F	Outlet Water Temperature °F	Tank Water Temperature °F	Exhaust Duct Temperature °F	Ambient Temperature °F
17	1.7	55	57	70	99	72	83
	1.7	55	57	73	100	70	82
			56	78	106	72	83
			57	82	110	75	83
			56	85	116	77	84
			57	87	121	79	83
			57	90	125	80	83
			57	92	129	82	82
			56	93	133	80	84
			56	96	138	81	83
			57	97	141	81	83
			57	99	144	82	83
			57	102	148	82	83
			57	102	150	83	93
			56	97	148	83	92
			57	94	145	81	92
			57	91	141	80	91
			56	90	138	80	92
			57	88	135	79	90
			56	87	132	79	91
			57	84	129	80	89
			56	83	127	82	87
	✓	✓	57	91	134	83	89

FURNACE OPERATING DATA

21

Cycle No.	Cooling Water Flowrate (gpm)	Thermostat Setting (°C)	Inlet Water Temperature (°F)	Outlet Water Temperature (°F)	Tank Water Temperature (°F)	Exhaust Duct Temperature (°F)	Ambient Temperature (°F)
	1.7	55	57	81	122	83	89
			56	77	119	83	87
			57	78	117	82	87
			56	77	115	81	85
			57	77	113	81	84
			57	76	111	81	83
			56	74	109	81	82
			56	73	107	81	82
			56	73	105	81	80
			56	72	104	80	82
			56	72	103	80	80
	✓		56	71	101	70	79
18	1.7	55	56	73	103	68	78
			56	78	108	72	77
			56	82	113	75	77
			56	85	118	76	77
			56	87	123	78	78
			56	90	128	78	78
			56	92	131	79	78
			56	94	136	80	79
			55	96	138	80	79
			56	98	143	80	77
	✓	✓	56	100	146	80	77

FURNACE OPERATING DATA

29

Cycle No.	Cooling Water Flowrate (gpm)	Thermostat Setting (°C)	Inlet Water Temperature °F	Outlet Water Temperature °F	Tank Water Temperature °F	Exhaust Duct Temperature °F	Ambient Temperature °F
	1.7	55	56	101	149	81	82
			56	104	151	82	81
			56	105	155	83	80
			56	106	157	83	83
			56	107	158	83	90
			55	103	158	83	91
			56	99	154	81	92
			56	96	150	80	90
			56	93	146	78	88
			56	91	143	77	89
			56	87	139	77	91
			56	87	136	77	83
			56	86	133	77	86
			56	83	130	77	85
			56	83	128	77	86
			56	81	125	77	86
			56	80	122	77	83
			56	79	120	77	81
			56	78	118	77	82
			56	77	116	77	79
			56	76	114	77	80
			56	76	112	77	82
	✓	✓	56	74	110	78	82

FURNACE OPERATING DATA

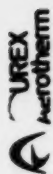
30

Cycle No.	Cooling Water Flowrate (gpm)	Thermostat Setting (°C)	Inlet Water Temperature °F	Outlet Water Temperature °F	Tank Water Temperature °F	Exhaust Duct Temperature °F	Ambient Temperature °F
	1.7	55	56	74	109	78	82
	↓		56	73	107	75	80
		↓	56	73	105	68	76
19	1.7	55	56	76	109	69	76
			56	81	114	74	78
			56	86	120	77	78
			56	89	124	80	78
			56	92	130	81	77
			56	95	134	82	80
			56	98	138	83	79
			56	100	142	84	80
			56	101	146	83	88
			56	96	145	82	90
			56	92	142	81	89
			56	90	138	80	87
			56	88	135	79	90
			56	86	131	79	87
			56	84	128	78	88
			56	83	126	77	85
			56	82	124	77	86
			56	80	121	77	86
			56	79	118	77	84
	✓	✓	56	78	117	76	82

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SECTION 4
SAMPLING DATA SHEETS



FIELD DATA SHEET

Plant RESIDENTIAL WATER HEATER WITH M.I.M. Burner
 Location ALBERTA COMMUNITY LABORATORY
 Operator CATALDINI
 Date 5-21-80
 Load Range CAPACITY
 Thermostat setting at 53.55°C
 Water flow rate 1.7 gpm

Sheet No. 1/8

Time	Sample Point	Load kW	Steam Flow Rate (kg/hr)	DRY UNCORRECTED						CORRECTED TO O ₂ = 13.5% OR DRY				HYDROCARBONS (HC)		Stack Gas Temp °F	COMMENTS
				O ₂ %	CO ₂ %	CO ppm	SO ₂ ppm	NO ppm	NO ₂ ppm	NO _x ppm	NO _x ppm	NO ₂ ppm	Total HC	Non-Hydrocarbon HC	H ₂ O %		
6:18	Exhaust			2.1	12.4	165	-	68	68	0			Not			60.7	Start of test - cycle No. 1
6:20	Duct			2.0	12.5	20		70	70	0			Burner			63.0	Burner on
6:22				1.9	12.7	15		72	72	0						73.3	Time = 12 min
6:24				1.85	12.7	15		72	72	0						76.3	
6:26				1.9	12.7	10		72	72	0						81.7	
6:28				1.85	12.7	10		72	72	0						83.4	Burner off
6:30																	
6:32																	
6:34																	
6:36				2.05		150		66	66	0						68	Start of Cycle No. 2
6:38				2.0		35		68	68	0						72.9	
6:40				2.0		35		70	70	0						72.3	Burner on
6:42				2.0		35		70	70	0						78.6	Time = 14 min
6:44				2.0		30		72	72	0						84.3	
6:46				2.1		30		72	72	0						84.3	
6:48				1.95		25		76.5	76.5	0						84.7	Burner off
6:50																	
6:52																	
6:54																	
6:56																	
6:58																	
7:00				2.2	17.3	150		68	68	0			1.5			62.1	Start of Cycle No. 3
7:02				2.13	12.7	25.2		68	68	0			1.2			76.7	
7:04				2.08	13.0	23.2		67.5	67.5	0			0.8			79.6	Burner on
7:06				2.05	13.3	24.7		70	70	0			1.0			80.8	Time = 8 min
7:08				2.07	12.5	24.4		67	67	0			0.9			82.1	
7:10				2.04	13.1	22.9		69	69	0			0.8			82.5	
7:12				2.02	12.7	24.8		70	70	0			0.7			83.8	AVERAGE

Form EED-083 12/78

e - AS C5M6

A UREX
Microtherm

Ambient Temperature	74-87 ° F Room air
Barometric Pressure	
Static Pressure Duct	
Fuel	Dust 112 k air

Sheet No. 2/3

[illegible] α A, C, Hg

Form EEO-013 12/79

UUREX
Aerotherm

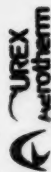
Ambient Temperature 73-83 Room Air
Barometric Pressure _____
Static Pressure Duct _____
Fuel D. 4112K 0.1

Sheet No. 3/8

[illegible]

2 - As C₆H₈

Form EED-063 12/76



FIELD DATA SHEET

Plant RESIDENTIAL WATER TREATMENT WITH H.A.N. Burner
Location Aluma Combustion Laboratory
Operator Cataldini
Date 5-21-80
Load Range Capacity
Thermostat Setting at 53-55°C
Water flow rate = 1.7 gpm
Ambient Temperature 71-83°F Room Air - close to
Barometric Pressure Burner
Static Pressure Duct
Fuel Distillate oil

Sheet No. 5 / 8

Time	Sample Point	Load MW	Steam Flow lb/hr x1000	DRY UNCORRECTED							CORRECTED TO 25°C, 50% O ₂ DRY				HYDROCARBONS (HC)			Stack Gas Temp °F	COMMENTS
				O ₂ %	CO ₂ %	CO ppm	SO ₂ ppm	NO ppm	NO ₂ ppm	NO _x ppm	NO ppm	NO ₂ ppm	NO _x ppm	Total HC & Methane	Non-Methane HC	H ₂ O %			
11:43	Exhaust			2.2	13.0	16.5		-	6.5	0				-			63.9	Start Cycle No. 10	
11:56	Duct			1.99	13.4	46.4		-	67.8					2.4			76		
11:58				1.96	13.3	44.7		-	70.3					1.0			79	Burner on	
12:00				1.93	13.0	41.7		-	71.3					0.7			81	Time = 13 min	
12:02				1.84	13.2	45.3		-	71.9					0.4			82		
12:06														-			82	Burner off	
12:29														-			64.3	Start Cycle No. 11	
12:30				2.0	12.2	155		-	66					2.0			66.4	Burner on	
12:32				1.97	12.5	38.6		-	67.6					2.0			73.1	Time = 13 min	
12:37				1.92	12.6	35.8		-	69.1					0.9			80.8		
12:42				1.65	12.9	36.3		-	73					-			83	Burner off	
				1.50	13.0	75		-	74					-					
13:08														-			66.6	Start Cycle No. 12	
13:09				1.95	12.5	150		-	66					-			70.6		
13:10				1.91	12.7	40.3		-	65					5.5			73.8	Burner on	
13:11				1.91	13.2	36		-	67.4					3.8			76.5	Time = 10 min	
13:12				1.83	12.9	34.8		-	69.4					2.6			77.3		
13:13				1.82	12.7	33.8		-	71.5					1.7			78.7		
13:14				1.73	13.0	34.5		-	70.7					2.3			79.2		
13:15				1.72	13.2	34.3		-	72.8					2.5			79.6		
13:16				1.63	12.8	34.7		-	73.3					-			81.7	Burner off	
13:18				1.45	13.0	38		-	75					-				AVERAGE	

Form EED-083 12/78

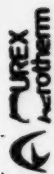
2 - Als C3H8

AUREX
Aurotherm

Sheet No. 6/8

[illegible]

Form EEO-003 12/78



FIELD DATA SHEET

Ambient Temperature 81 - 83 °F Room Air 60 to Bureau
Barometric Pressure _____
Static Pressure Duct Discharge Oil
Fuel _____

Plant Reactor: Water Heater with MAF Burner
Location Process Combustion Laboratory
Operator CHAD DILL
Date 5-21-89
Load Range Capacity ~ 2.51 gph
Thermocouple Setting 54°C
Water Flow rate = 1.7 gpm

Sheet No. 7/8

Time	Sample Point	Load MW	Steam Flow Rate (1000)	DRY UNCORRECTED							CORRECTED TO 0% O ₂ DRY				HYDROCARBONS (HC)			H ₂ O %	Stack Gas Temp °F	COMMENTS
				O ₂ %	CO ₂ %	CO ppm	SO ₂ ppm	NO ppm	NO _x ppm	NO ₂ ppm	NO ppm	NO _x ppm	NO ₂ ppm	Total HCs	Methane	Non-Methane HC				
15:37	Exhaust			2.59	12.8	155		-	67.7	0				5.2			71	Start Cycle No. 16		
15:40	Duct			2.02	13.0	29.8		-	68.0					3.7			76			
15:42				1.97	12.6	28.8		-	67.7					3.2			80.1	Burner on line		
15:44				1.86	13.0	24.7		-	71.0					1.9			82.4	= 12 hrs		
15:46				1.81	13.0	23.5		-	71.8					2.3			81.7			
15:48				1.65	13.2	26		-	68.5					-			82.5	Burner off		
15:49				1.50	13.4	25		-	71.2					-			83.7			
16:13				2.0	12.5	175		-	67.5					-			69.7	Start Cycle No. 17		
16:15				1.98	12.7	15.2		-	66.2					6.1			75.1			
16:17				1.90	13.2	14.3		-	69.9					5.9			73.7	Burner on		
16:19				1.84	13.1	10.7		-	71.1					4.6			78.6	line = 12		
16:21				1.70	13.1	11.0		-	73.2					3.9			80.1			
16:23				1.58	12.5	12.5		-	74.5					3.2			81.2	CO calibration off		
16:25				1.45	13.6	18		-	75.0					-			81.5			
16:47				1.95	12.5	155		-	68					-			68.4	Start Cycle No. 18		
16:50				1.67	12.6	38		-	68.4					4.4			76	Burner on		
16:52				1.90	12.8	37.6		-	72					2.0			79.3	line = 16 hrs		
16:54				1.64	13.0	32.4		-	72.9					2.3			78.6			
16:56				1.44	13.2	37.5		-	74					-			80.4	Burner off		
17:03				1.45	13.2	30		-	76					-			83.4			
																		AVERAGE		

Form EED-003 12/78

0 91 C3H8

FAUREX
Microtherm

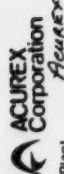
Ambient Temperature	31-33 °F	Room Temp
Barometric Pressure	(close to 30 in)	
Static Pressure Duct		
Fuel	Distillate oil	

Sheet No. 3/8

[illegible]

2 - As C 45

Form EED-063 12/79



ACUREX Corporation
HENKEY

Plant

Date

Test Location

Run Number

Stack Diameter inches

Duct Dimensions in. x in.

Start Time

Operator

Page 1 of 4

Barometric Pressure

Static Pressure

Stack Pressure

Probe Number

Pilot Coefficient

Pilot Number

Meter Box Number

Orifice Coefficient

SASS PARTICULATE TEST FIELD DATA SHEET

Nozzle Size & Number

Molecular Weight

BWO

IMPINGER VOLUMES

TIME

CO₂

O₂

CO

NUMBER

TARE

FINAL WT

SILICA GEL

750

TEMPERATURES °F

IMPINGER

ORGANIC MODULE

OVEN

GAS METER

IN

OUT

PUMP VACUUM

in Hg

√SP

LEAK RATE

in Hg

STACK

PROBE

IMPINGER

ORGANIC MODULE

OVEN

GAS METER

IN

OUT

PUMP VACUUM

in Hg

√SP

LEAK RATE

in Hg

GAS METER VOLUME FT³

ORIFICE METER

VELOCITY HEAD

CLOCK TIME

SAMPLE POINT

STOP

START

STOP

START

STOP

START

STOP

START

STOP

START

STOP

START

STOP

START

STOP

START

STOP

START

STOP

START

STOP

START

STOP

START

STOP

START

STOP

START

STOP

START

STOP

START

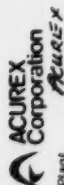
STOP

START

STOP

START

STOP



ACUREX
Corporation

PAGE 2 of 4

GAUSS PARTICULATE TEST FIELD DATA SHEET

Plant

Date

5-21-80

Barometric Pressure

Test Location

Static Pressure

Stack Pressure

Run Number

Probe Number

Pilot Coefficient

Stack Diameter inches

Pilot Coefficient

Pilot Number

Duct Dimensions in. x in.

Meter Box Number

Operator

Start Time

Orifice Coefficient

Nozzle Size & Number

Molecular Weight

BWO

IMPINGING VOLUMES

TIME

CO₂

O₂

CO

SILICA GEL

NUMBER

TARE

FINAL WT

TEMPERATURES °F

IMPINGING

ORGANIC MODULE

OVEN

GAS METER

IN

OUT

PUMP VACUUM

in Hg

√P

CHANGES

80-100

77 mH

CHANGES

80-100

77 mH

CHANGES

1002

STOP

START

STOP

START

STOP

START

1040

STOP

START

STOP

START

STOP

START

1118

STOP

START

STOP

START

STOP

START

1154

STOP

START

STOP

START

STOP

START

AVG TOTAL

138.3

138.3

138.3

138.3

138.3

138.3

Plant	Barometric Pressure	Nozzle Size & Number	IMPINGER VOLUMES	TIME	CO ₂	O ₂	CO
Auto-X	Static Pressure	Molecular Weight					
Date 5-17-80	Stack Pressure	BWO					
Test Location FURNACE MAN RIS	Probe Number						
Run Number							
Stack Diameter inches	Pilot Coefficient						
Duct Dimensions in. x in.	Pilot Number						
Start Time	Meter Box Number						
Separator	Orifice Coefficient						

[illegible]

SECTION 5
ANALYTICAL LABORATORY RESULTS

5.1 FUEL ANALYSIS REPORT

LABORATORY CERTIFICATE

CURTIS & TOMPKINS, LTD.

ESTABLISHED 1878

ANALYTICAL - CHEMISTS - CONSULTING

SAMPLERS - INSPECTORS

MEMBERS OFFICIAL CHEMISTS
AND/OR SAMPLERS FOR MANY
COMMODITY AND TRADE ORGANIZATIONS

CORPORATE OR STAFF MEMBERSHIP IN
PRINCIPAL SCIENTIFIC SOCIETIES

280 DIVISION STREET
SAN FRANCISCO, CALIF. 94103
U.S.A.

CABLE ADDRESS ANALYST

REFEREE ANALYSES
RESEARCH - INVESTIGATIONS
VITAMIN ASSAYS - BIOCHEMISTRY

SPECIALISTS IN BULK COMMODITIES

Laboratory No. 80k10
Preliminary No. 4132

Reported 10/6/80
Sampled -----
Received 9/8/80

For ACUREX CORPORATION

Report on 5 samples of Oil

Mark	<u>Sample #</u>	<u>Sample Type</u>
	(5) 80-1562,	Fuel Oil
	(All) Project No.: 7601.22, Customer No.: RB 59186A, rel. 01,	
	Prime Contract: 68-02, 3188, Acurex Project: 7601, Date:	
	September 5, 1980, Subcontract No.: RB59186A.	

	(5)		
	1st	2nd	3rd
	<u>Test</u>	<u>Test</u>	<u>Test</u>
Carbon (C), % -----	86.94	----	----
Hydrogen (H), % ---	12.90	----	----
Oxygen (O), by			
difference, % -----	0.01*	----	----
Nitrogen (N), % ---	0.03	0.04	0.05
Sulfur (S), % -----	0.18	0.20	0.20
Heating Value:			
BTU/Pound -----	19,190	----	----
Gravity,			
°API @ 60°F -----	33.75	----	----

*Less Than

Curtis & Tompkins Ltd.

5.2 PARTICULATE GRAVIMETRIC ANALYSES AND MASS EMISSION CALCULATIONS

PARTICULATE CALCULATIONS

FOR HIGH VOLUME STACK SAMPLER - METHOD 5

1. Volume of dry gas sampled at standard conditions, 68°F, 29.92 inch Hg (scf)

$$Vm_{std} = 17.64 \frac{Vm}{\alpha} \left(\frac{P_b + \frac{\Delta H}{13.6}}{T_{m,avg} + 460} \right) = 17.64 \left(\frac{350.517}{1.013} \right) \left(\frac{29.87 + 0.77/13.6}{543} \right)$$

$$\text{or } Vm_{stack} = \left(\frac{Vm}{\alpha} + Vw_{std} \right) \left(\frac{T_{s,avg} + 460}{T_{m,avg} + 460} \right) \left(\frac{P_m}{P_s} \right) \quad (\text{acf at stack conditions})$$

$$= 336.4$$

2. Stack gas moisture condensed at standard conditions (scf)

$$Vw_{std} = 0.04707 V_{Ic}$$

$$= 0.04707 (199.9) = 9.409$$

3. Stack gas proportion of water vapor, by volume

$$B_{wo} = \frac{Vw_{std}}{Vw_{std} + Vm_{std}}$$

$$= \frac{9.409}{9.409 + 336.4} = 2.7\%$$

4. Stack gas dry molecular weight (lb/lb-mole)

$$MW_d = 0.44 (\%CO_2) + 0.32 (\%O_2) + 0.28 (\%N_2 + \%CO)$$

$$= 0.44 (12.9) + 0.32 (1.9) + 0.28 (85.2) = 30.14$$

5. Stack gas molecular weight (lb/lb-mole)

$$MW_s = MW_d (1 - B_{wo}) + 18 (B_{wo})$$

$$= 30.14 (1 - 0.027) + 18 (0.027) = 29.81$$

6. Pressure stack, in. Hg

$$P_s = P_b + \frac{P_{st}}{13.6}$$

$$= 29.89$$

7. Stack gas velocity at stack conditions (ft/sec)

$$V_s = 85.49 (C_p) (\sqrt{\Delta P})_{avg} \sqrt{\frac{T_{s,avg} + 460}{P_s MW_s}}$$

$$= 200 \text{ ft/min} = 3.3 \text{ ft/s}$$

Velocity was not detectable with pitot tube or inclined water manometer. Stack gas velocity was measured with hot wire anemometer.

8. Stack gas volume at standard conditions (scfm)

$$Q_s = 60 (1 - B_{wo}) V_{s,avg} A_s \left(\frac{528}{T_{s,avg} + 460} \right) \left(\frac{P_s}{29.92} \right) =$$

$$\text{or } Q_s = 60 V_{s,avg} A_s (\text{scfm}) = 60 (1 - 0.027) (3.3) \frac{\pi}{4} (144) \left(\frac{528}{75 + 460} \right) \frac{29.89}{29.92} = 16.72 \text{ cfs}$$

9. Test percent isokinetic

$$\%I = \frac{17.33 (T_{s,avg} + 460) [0.04707 (W_{ic}) + V_{m,std}]}{0 V_s P_s D_n^2}$$

$$= 104.4 \%$$

10. Particulate matter concentration, gr/scf

$$C_s = 15.432 \frac{M_p}{V_{m,std}} = \frac{15.432 (0.0175 \text{ grams})}{336.4} = 8.028 \times 10^{-4}$$

$$\text{or } C_s = 15.432 \frac{M_p}{V_{m,stack}} (\text{gr/scf}) =$$

11. Emission rate of particulate matter, lb/hr

$$ER = 0.00857 (Q_s) C_s$$

$$= 0.00857 (16.72) (8.028 \times 10^{-4}) = 1.15 \times 10^{-4} \text{ lb/hr}$$

$$= 0.0063 \text{ lb}/10^6 \text{ Btu}$$

12. Percent excess air at sampling point

$$= 2.70 \%$$

$$\%EA = \frac{100 [\%O_2 - 0.5 (\%CO)]}{0.264 \%N_2 - (\%O_2 - 0.5 \times \%CO)} =$$

13. Emission rate of particulate matter, lb/10⁶ Btu

$$E = 2.679 \times 10^4 \left(\frac{M_p}{V_{m,std}} \right) F \left(\frac{20.9}{20.9 - \%O_2} \right)$$

=

PARTICULATE CALCULATIONS

1. Volume of dry gas sampled at standard conditions, 68°F, 29.92 inch Hg (scf)

$$V_{m_{std}} = 17.64 \frac{V_m}{\alpha} \left(\frac{P_b + \frac{\Delta H}{13.6}}{T_{m,avg} + 460} \right) = 17.64 \frac{1031.864}{1,003} \left(\frac{29.87 + \frac{4}{13.6}}{544} \right) = 1006.263$$

$$\text{or } V_{m_{stack}} = \left(\frac{V_m}{\alpha} + V_{w_{std}} \right) \left(\frac{T_{s,avg} + 460}{T_{m,avg} + 460} \right) \left(\frac{P_m}{P_s} \right) \quad (\text{acf at stack conditions})$$

=

2. Stack gas moisture condensed at standard conditions (scf)

$$V_{w_{std}} = 0.04707 V_{I_c}$$

$$= 31.311$$

3. Stack gas proportion of water vapor, by volume

$$B_{wo} = \frac{V_{w_{std}}}{V_{w_{std}} + V_{m_{std}}}$$

$$= \frac{31.311}{1006.263 + 31.311} = 3.0\%$$

4. Stack gas dry molecular weight (lb/lb-mole)

$$MW_d = 0.44 (\%CO_2) + 0.32 (\%O_2) + 0.28 (\%N_2 + \%CO)$$

$$= 30.14$$

5. Stack gas molecular weight (lb/lb-mole)

$$MW_s = MW_d (1 - B_{wo}) + 18 (B_{wo})$$

$$= 30.14 (1 - 3.0) + 18 (3.0)$$

6. Pressure stack, in. Hg

$$P_s = P_b + \frac{P_{st}}{13.6}$$

$$= 29.89$$

7. Stack gas velocity at stack conditions (ft/sec)

$$V_s = 85.49 (C_p) (\sqrt{\Delta P})_{avg} \sqrt{\frac{T_{s,avg} + 460}{P_s MW_s}}$$

$$= \sim 200 \text{ ft/min} \approx 3.3 \text{ ft/s}$$

Velocity was too small to be detected with pitot tube or inclined water manometer. Stack gas velocity was measured with hot wire anemometer.

8. Stack gas volume at standard conditions (scfm)

$$Q_s = 60 (1 - B_{wo}) V_{s,avg} A_s \left(\frac{528}{T_{s,avg} + 460} \right) \left(\frac{P_s}{29.92} \right) =$$

$$\text{or } Q_s = 60 V_{s,avg} A_s (\text{acfm}) = 60 (1.003) 3.3 \frac{\pi}{4} 4^2 \frac{528}{75+460} \frac{29.92}{29.92} = 16.53$$

9. Test percent isokinetic

$$\%I = \frac{17.33 (T_{s,avg} + 460) [0.04707 (W_{ic}) + V_{m,std}]}{\theta V_s P_s D_n^2}$$

$$= 17.2 \%$$

10. Particulate matter concentration, gr/scf

$$C_s = 15.432 \frac{M_p}{V_{m,std}} = 15.432 \frac{0.0240}{1006.26} = 0.0004$$

$$\text{or } C_a = 15.432 \frac{M_p}{V_{m,stack}} (\text{gr/acf}) =$$

11. Emission rate of particulate matter, lb/hr

$$ER = 0.00857 (Q_s) C_s$$

$$= 0.00857 (16.53) (0.0004) = 0.0001 \text{ lb/hr}$$

$$= 0.0029 \text{ lb/10}^6 \text{ Btu}$$

$$= 1.22 \text{ ug/J}$$

12. Percent excess air at sampling point

$$\% EA = \frac{100 [\%O_2 - 0.5 (\%CO)]}{0.264 \%N_2 - (\%O_2 - 0.5 \%CO)} =$$

13. Emission rate of particulate matter, lb/10⁶ Btu

$$E = 2.679 \times 10^4 \left(\frac{M_p}{V_{m,std}} \right) F \left(\frac{20.9}{20.9 - \%O_2} \right)$$

=

5.3 SULFUR ANALYSIS REPORT AND EMISSION CALCULATION

H₂SO₄ Mist and SO₂
Clean-Up and Analysis

Plant M.A.N. Residential Furnace

Date 5-21-80

Project No. CHEA 7605.21

Recorded by G. Nicoll

H₂SO₄ Mist and SO₂ Train Description and Recovery Data

Impinger Sequence	1	2	3	4	5	6
Impinger Number	—	—	—	—	—	—
G-S Standard ()	—	—	—	—	—	—
G-S Modified ()	—	—	—	—	—	—
Contents	<u>IPA</u>	<u>H₂O₂</u>	<u>H₂O₂</u>	<u>Empty</u>	<u>S. O₂</u>	—
Concentration (%)	<u>80</u>	<u>3</u>	<u>3</u>	—	—	—
Initial Volume (ml)	<u>100</u>	<u>100</u>	<u>100</u>	<u>0</u>	<u>250.00</u>	—
Final Volume (ml)	<u>55</u>	<u>115</u>	<u>115</u>	<u>3</u>	<u>276.00</u>	—
Dilution Volume (ml)	<u>250</u>	<u>500</u>	—	—	—	—
Container Number	<u>102</u>	<u>103</u>	<u>103</u>	—	—	—

Location of glass wool in train Between impingers 1 & 2

Location and type of filter in train glass filter

Total Condensate

(No. 8-7) 45 ml

$$C_{SO_2} = 7.05 \times 10^{-5} (19.4 - 0.05) 0.011 \left(\frac{500}{5} \right) = 2.34 \times 10^{-5} \frac{16}{11}$$

H₂O₂ Sample Blank No. 104

IPA Sample Blank No. 101

$C_{SO_2} = 0$

H₂SO₄ and SO₂ Analysis

Sample Number	<u>102</u>	<u>103</u>				
Volume of Titrant for Sample (ml)	<u>0.1</u>	<u>19.4</u>				
Volume of Titrant for Blank (ml)	<u>0.1</u>	<u>0.05</u>				
Normality of Titrant	<u>0.011</u>	<u>0.011</u>				
Ttl. Vol. of Soln. (ml)	<u>250</u>	<u>250</u>				
Volume of Sample Aliquot Titrated (ml)	<u>100</u>	<u>510</u>				
Performed by	<u>Gerda Vanden</u>					

Spike and recovery at start Interference 103% Solution past end point

5.4 TRACE ELEMENT ANALYSIS REPORT

COMMERCIAL TESTING & ENGINEERING CO.

GENERAL OFFICES: 222 NORTH LA SALLE STREET, CHICAGO, ILLINOIS 60601 - AREA CODE 312 759-9434
INSTRUMENTAL ANALYSIS DIVISION, 14235 WEST 46TH AVENUE, GOLDEN, COLORADO 80401, PHONE: 303-276-9521

Reply to

To: Mr. Brent Higginbotham
Acurex Corporation
485 Clyde Ave.
Mountain View, CA 94042



Date: September 23, 1980

Analyst: T. Bouts

P. O. No.:

Sample No.: 80-1535

XAD-BLANK

SPARK SOURCE MASS SPECTROGRAPHIC ANALYSIS
CONCENTRATION IN PPM WEIGHT

IAD No.: 97-E697-116-28

ELEMENT	CONC.	ELEMENT	CONC.	ELEMENT	CONC.	ELEMENT	CONC.
Uranium		Terbium		Ruthenium		Vanadium	0.3
Thorium		Gadolinium		Molybdenum	0.4	Titanium	2
Bismuth	<0.1	Europium		Niobium		Scandium	<0.1
Lead	0.6	Samarium		Zirconium	1	Calcium	170
Thallium		Neodymium		Yttrium	<0.1	Potassium	24
Mercury	NR	Praseodymium	0.2	Strontium	0.2	Chlorine	1
Gold		Cerium	0.9	Rubidium	<0.1	Sulfur	9
Platinum		Lanthanum	2	Bromine	0.4	Phosphorus	26
Iridium		Barium	1	Selenium	0.2	Silicon	*MC
Osmium		Cesium		Arsenic	<0.1	Aluminum	13
Rhenium		Iodine	<0.1	Germanium	0.2	Magnesium	17
Tungsten		Tellurium		Gallium	0.1	Sodium	98
Tantalum	≤0.6	Antimony	0.2	Zinc	6	Fluorine	=3
Hafnium		Tin	0.1	Copper	13	Oxygen	NR
Lutetium		Indium	STD	Nickel	MC	Nitrogen	NR
Ytterbium		Cadmium		Cobalt	3	Carbon	NR
Thulium		Silver		Iron	120	Boron	<0.1
Erbium		Palladium		Manganese	39	Beryllium	
Holmium		Rhodium		Chromium	29	Lithium	0.1
Dysprosium						Hydrogen	NR

* Heterogeneous

STD - Internal Standard

NR - Not Reported

All elements not detected < 0.1 ppm

MC - Major Component

INT - Interference

Approved:

M. J. Speck

COMMERCIAL TESTING & ENGINEERING CO.

GENERAL OFFICES: 898 NORTH LA SALLE STREET, CHICAGO, ILLINOIS 60601 • AREA CODE 312 788-8434
INSTRUMENTAL ANALYSIS DIVISION, 14235 WEST 44TH AVENUE, GOLDEN, COLORADO 80401, PHONE: 303-578-9521

Reply to

To: Mr. Brent Higginbotham
Acurex Corporation
485 Clyde Avenue
Mountain View, California 94042



Date: September 26, 1980

Analyst: T. Bouts

P. O. No.:

Sample No.: 1475

SPARK SOURCE MASS SPECTROGRAPHIC ANALYSIS

IAD No.: 97-E697-116-28

MAN TEST
1st IMPINGER BLANK

CONCENTRATION IN $\mu\text{g/ml}$

ELEMENT	CONC.	ELEMENT	CONC.	ELEMENT	CONC.	ELEMENT	CONC.
Uranium		Terbium		Ruthenium		Vanadium	0.002
Thorium		Gadolinium		Molybdenum	0.05	Titanium	0.01
Bismuth		Europium		Niobium		Scandium	0.001
Lead	≤ 0.02	Samarium		Zirconium	0.01	Calcium	6
Thallium		Neodymium		Yttrium		Potassium	2
Mercury	NR	Praseodymium		Strontium	0.006	Chlorine	0.3
Gold		Cerium		Rubidium		Sulfur	0.2
Platinum		Lanthanum		Bromine	0.02	Phosphorus	3
Iridium		Barium	* 1	Selenium		Silicon	0.4
Osmium		Cesium		Arsenic		Aluminum	0.3
Rhenium		Iodine	0.01	Germanium		Magnesium	0.08
Tungsten		Tellurium		Gallium		Sodium	* 6
Tantalum		Antimony		Zinc	0.07	Fluorine	=0.1
Hafnium		Tin	0.2	Copper	0.07	Oxygen	NR
Lutetium		Indium	STD	Nickel	0.03	Nitrogen	NR
Ytterbium		Cadmium		Cobalt	0.004	Carbon	NR
Thulium		Silver	0.5	Iron	0.09	Boron	0.001
Erbium		Palladium		Manganese	0.006	Beryllium	
Holmium		Rhodium		Chromium	0.006	Lithium	0.002
Dysprosium						Hydrogen	NR

STD - Internal Standard

NR - Not Reported

All elements not detected < $0.004 \mu\text{g/ml}$

MC - Major Component > $10 \mu\text{g/ml}$

INT - Interference

Approved:

* Heterogeneous

M. Jacobs

COMMERCIAL TESTING & ENGINEERING CO.

GENERAL OFFICES: 222 NORTH LA SALLE STREET, CHICAGO, ILLINOIS 60601 - AREA CODE 312 739-6434
INSTRUMENTAL ANALYSIS DIVISION, 14325 WEST 44TH AVENUE, GOLDEN, COLORADO 80401, PHONE: 303-378-9521

Reply to

To: Mr. Brent Higginbotham
Acurex Corporation
485 Clyde Ave.
Mountain View, CA 94042



Date: September 25, 1980

Analyst: T. Bouts

P. O. No.:

Sample No.: A-45 Blank SPARK SOURCE MASS SPECTROGRAPHIC ANALYSIS IAD No.: 97-E697-116-28

Filter Blank

CONCENTRATION IN $\mu\text{g}/\text{cm}^2$

ELEMENT	CONC.	ELEMENT	CONC.	ELEMENT	CONC.	ELEMENT	CONC.
Uranium	<0.008	Terbium		Ruthenium		Vanadium	0.02
Thorium	0.01	Gadolinium		Molybdenum	0.005	Titanium	0.9
Bismuth		Europium		Niobium	0.01	Scandium	0.002
Lead	0.007	Samarium		Zirconium	0.05	Calcium	MC
Thallium		Neodymium	0.004	Yttrium	0.003	Potassium	0.5
Mercury	NR	Praseodymium	0.003	Strontium	0.04	Chlorine	0.4
Gold	<0.001	Cerium	0.01	Rubidium	<0.001	Sulfur	0.8
Platinum		Lanthanum	0.02	Bromine	0.03	Phosphorus	0.07
Iridium		Barium	0.2	Selenium	0.005	Silicon	MC
Osmium		Cesium		Arsenic	0.001	Aluminum	MC
Rhenium		Iodine	0.001	Germanium		Magnesium	1
Tungsten	0.01	Tellurium		Gallium	0.008	Sodium	>2
Tantalum		Antimony		Zinc	0.01	Fluorine	=0.005
Hafnium		Tin		Copper	0.01	Oxygen	NR
Lutetium		Indium	STD	Nickel	0.01	Nitrogen	NR
Ytterbium		Cadmium		Cobalt	<0.001	Carbon	NR
Thulium		Silver	0.001	Iron	0.2	Boron	0.9
Erbium		Palladium		Manganese	0.003	Beryllium	
Holmium		Rhodium		Chromium	0.02	Lithium	<0.001
Dysprosium						Hydrogen	NR

STD - Internal Standard

NR - Not Reported

All elements not detected <0.001 $\mu\text{g}/\text{cm}^2$

MC - Major Component > 10 $\mu\text{g}/\text{cm}^2$

INT - Interference

Approved:

M. J. Speck

COMMERCIAL TESTING & ENGINEERING CO.

GENERAL OFFICES: 830 NORTH LA SALLE STREET, CHICAGO, ILLINOIS 60601 • AREA CODE 312 738-8434
INSTRUMENTAL ANALYSIS DIVISION, 14335 WEST 44TH AVENUE, GOLDEN, COLORADO 80401, PHONE: 303-375-9321

Reply to

To: Mr. Brent Higginbotham
Acurex Corporation
485 Clyde Ave.
Mountain View, CA 94042



Date: September 24, 1980

Analyst: L. Jacobs

P. O. No.:

Sample No.: 80-1312

TAP WATER BLANK

SPARK SOURCE MASS SPECTROGRAPHIC ANALYSIS
CONCENTRATION IN $\mu\text{g/ml}$

IAD No.: 97-E697-116-28

ELEMENT	CONC.	ELEMENT	CONC.	ELEMENT	CONC.	ELEMENT	CONC.
Uranium		Terbium		Ruthenium		Vanadium	<0.001
Thorium		Gadolinium		Molybdenum	0.1	Titanium	0.1
Bismuth		Europium		Niobium	0.005	Scandium	
Lead	0.01	Samarium		Zirconium	0.02	Calcium	10
Thallium		Neodymium		Yttrium		Potassium	0.05
Mercury		Praseodymium		Strontium	0.02	Chlorine	MC
Gold		Cerium		Rubidium	≤ 0.002	Sulfur	0.4
Platinum		Lanthanum		Bromine	0.1	Phosphorus	0.9
Iridium		Barium	0.02	Selenium		Silicon	0.2
Osmium		Cesium		Arsenic	0.004	Aluminum	0.004
Rhenium		Iodine		Germanium		Magnesium	MC
Tungsten		Tellurium		Gallium	0.009	Sodium	0.2
Tantalum		Antimony		Zinc	0.04	Fluorine	=0.004
Hafnium		Tin		Copper	0.002	Oxygen	NR
Lutetium		Indium	STD	Nickel	0.003	Nitrogen	NR
Ytterbium		Cadmium		Cobalt	$*\leq 0.002$	Carbon	NR
Thulium		Silver		Iron	0.2	Boron	<0.001
Erbium		Palladium		Manganese	0.006	Beryllium	
Holmium		Rhodium		Chromium	0.07	Lithium	INT
Dysprosium						Hydrogen	NR

* Heterogeneous

STD - Internal Standard

NR - Not Reported

All elements not detected < 0.002 $\mu\text{g/ml}$

MC - Major Component > 10 $\mu\text{g/ml}$

INT - Interference

Approved:

M. Jacobs

COMMERCIAL TESTING & ENGINEERING CO.

GENERAL OFFICES: 838 NORTH LA SALLE STREET, CHICAGO, ILLINOIS 60611 • AREA CODE 312 736-8434
INSTRUMENTAL ANALYSIS DIVISION, 14325 WEST 46TH AVENUE, GOLDEN, COLORADO 80401, PHONE: 303-378-9521

Reply to

To: Mr. Brent Higginbotham
Acurex Corporation
485 Clyde Ave.
Mountain View, CA 94042



Date: September 24, 1980

Analyst: T. Bouts

P. O. No.:

Sample No.: 80-1313

WATER TANK BLANK

SPARK SOURCE MASS SPECTROGRAPHIC ANALYSIS
CONCENTRATION IN $\mu\text{g/ml}$

IAD No.: 97-E697-116-28

ELEMENT	CONC.	ELEMENT	CONC.	ELEMENT	CONC.	ELEMENT	CONC.
Uranium		Terbium		Ruthenium		Vanadium	0.003
Thorium		Gadolinium		Molybdenum	*0.1	Titanium	0.1
Bismuth		Europium		Niobium		Scandium	<0.001
Lead	0.03	Samarium		Zirconium	0.02	Calcium	10
Thallium		Neodymium		Yttrium		Potassium	2
Mercury	NR	Praseodymium		Strontium	0.03	Chlorine	8
Gold		Cerium		Rubidium	0.006	Sulfur	2
Platinum		Lanthanum		Bromine	0.3	Phosphorus	0.4
Iridium		Barium	0.2	Selenium		Silicon	2
Osmium		Cesium		Arsenic	0.002	Aluminum	0.2
Rhenium		Iodine	0.01	Germanium		Magnesium	0.5
Tungsten		Tellurium		Gallium	≤ 0.007	Sodium	2
Tantalum		Antimony		Zinc	*2	Fluorine	=0.1
Hafnium		Tin		Copper	4	Oxygen	NR
Lutetium		Indium	STD	Nickel	0.04	Nitrogen	NR
Ytterbium		Cadmium		Cobalt	0.005	Carbon	NR
Thulium		Silver		Iron	0.3	Boron	0.002
Erbium		Palladium		Manganese	0.01	Beryllium	
Holmium		Rhodium		Chromium	0.008	Lithium	0.004
Dysprosium						Hydrogen	NR

* Heterogeneous

STD - Internal Standard

NR - Not Reported

All elements not detected < 0.003 $\mu\text{g/ml}$

MC - Major Component > 10 $\mu\text{g/ml}$

INT - Interference

Approved:

M. J. Jacobs

COMMERCIAL TESTING & ENGINEERING CO.

GENERAL OFFICES: 330 NORTH LA SALLE STREET, CHICAGO, ILLINOIS 60601 · AREA CODE 312 728-8434
INSTRUMENTAL ANALYSIS DIVISION, 14225 WEST 46TH AVENUE, GOLDEN, COLORADO 80401, PHONE 303-778-9221

Reply to

To: Mr. Brent Higginbotham
Acurex Corporation
485 Clyde Ave.
Mountain View, CA 94042



Date: September 24, 1980

Analyst: T. Bouts

P. O. No.:
Sample No.: 80-1368
TANK SAMPLE PRIOR
TO TEST

SPARK SOURCE MASS SPECTROGRAPHIC ANALYSIS
CONCENTRATION IN $\mu\text{g/ml}$

IAD No.: 97-E697-116-28

ELEMENT	CONC.	ELEMENT	CONC.	ELEMENT	CONC.	ELEMENT	CONC.
Uranium		Terbium		Ruthenium		Vanadium	0.007
Thorium		Gadolinium		Molybdenum	*0.4	Titanium	0.3
Bismuth		Europium		Niobium		Scandium	0.004
Lead	0.1	Samarium		Zirconium	0.05	Calcium	MC
Thallium		Neodymium		Yttrium	0.05	Potassium	0.5
Mercury	NR	Praseodymium		Strontium	0.05	Chlorine	0.4
Gold		Cerium	0.008	Rubidium	<0.001	Sulfur	MC
Platinum		Lanthanum	0.01	Bromine	0.4	Phosphorus	0.4
Iridium		Barium	0.07	Selenium	0.1	Silicon	1
Osmium		Cesium		Arsenic	INT	Aluminum	0.1
Rhenium		Iodine		Germanium	0.002	Magnesium	0.6
Tungsten	0.02	Tellurium	0.02	Gallium	0.004	Sodium	0.8
Tantalum		Antimony		Zinc	MC	Fluorine	*=0.2
Hafnium		Tin		Copper	MC	Oxygen	NR
Lutetium		Indium	STD	Nickel	1	Nitrogen	NR
Ytterbium		Cadmium		Cobalt	0.02	Carbon	NR
Thulium		Silver	0.03	Iron	MC	Boron	0.002
Erbium		Palladium		Manganese	0.2	Beryllium	
Holmium		Rhodium		Chromium	0.7	Lithium	0.002
Dysprosium						Hydrogen	NR

* Heterogeneous

STD - Internal Standard
NR - Not Reported
All elements not detected < 0.002 $\mu\text{g/ml}$
MC - Major Component > 10 $\mu\text{g/ml}$
INT - Interference

Approved:

M. J. Jacobs

COMMERCIAL TESTING & ENGINEERING CO.

GENERAL OFFICES: 888 NORTH LA SALLE STREET, CHICAGO, ILLINOIS 60601 • AREA CODE 312 788-8434
INSTRUMENTAL ANALYSIS DIVISION, 14333 WEST 44TH AVENUE, GOLDEN, COLORADO 80401, PHONE: 303-278-9521

Reply to

To: Mr. Brent Higginbotham
Acurex Corporation
485 Clyde Avenue
Mountain View, California 94042



Date: September 26, 1980

Analyst: T. Bouts

P. O. No.:

Sample No.: 1471
MAN TEST
FILTER

SPARK SOURCE MASS SPECTROGRAPHIC ANALYSIS
CONCENTRATION IN $\mu\text{g}/\text{cm}^2$

IAD No.: 97-E697-116-28

ELEMENT	CONC.	ELEMENT	CONC.	ELEMENT	CONC.	ELEMENT	CONC.
Uranium		Terbium		Ruthenium		Vanadium	0.2
Thorium		Gadolinium		Molybdenum	0.05	Titanium	1
Bismuth		Europium		Niobium	0.005	Scandium	≤ 0.001
Lead	0.2	Samarium		Zirconium	0.006	Calcium	MC
Thallium		Neodymium	0.005	Yttrium	0.008	Potassium	0.5
Mercury	NR	Praseodymium	0.003	Strontium	0.08	Chlorine	0.3
Gold		Cerium	0.03	Rubidium	< 0.001	Sulfur	8
Platinum		Lanthanum	0.04	Bromine	0.04	Phosphorus	0.5
Iridium		Barium	* 0.9	Selenium		Silicon	MC
Osmium		Cesium		Arsenic	≤ 0.001	Aluminum	MC
Rhenium		Iodine		Germanium	≤ 0.006	Magnesium	4
Tungsten		Tellurium		Gallium	0.009	Sodium	MC
Tantalum		Antimony		Zinc	0.6	Fluorine	=1
Hafnium		Tin	0.02	Copper	0.3	Oxygen	NR
Lutetium		Indium	STD	Nickel	0.04	Nitrogen	NR
Ytterbium		Cadmium		Cobalt	0.02	Carbon	NR
Thulium		Silver	0.002	Iron	0.7	Boron	MC
Erbium		Palladium		Manganese	0.02	Beryllium	
Holmium		Rhodium		Chromium	0.1	Lithium	0.02
Dysprosium						Hydrogen	NR

STD - Internal Standard
NR - Not Reported
All elements not detected $< 0.001 \mu\text{g}/\text{cm}^2$
MC - Major Component $> 10 \mu\text{g}/\text{cm}^2$
INT - Interference

Approved:

M. J. Speer

* Heterogeneous

COMMERCIAL TESTING & ENGINEERING CO.

GENERAL OFFICES: 938 NORTH LA SALLE STREET, CHICAGO, ILLINOIS 60601 • AREA CODE 312 798-9434
INSTRUMENTAL ANALYSIS DIVISION, 14335 WEST 44TH AVENUE, GOLDEN, COLORADO 80401, PHONE: 303-279-9531

Reply to

To: Mr. Brent Higginbotham
Acurex Corporation
485 Clyde Avenue
Mountain View, California 94042



Date: September 26, 1980

Analyst: T. Bouts

P. O. No.:

Sample No.: 1476

MAN TEST

1st IMPINGER + OMC

SPARK SOURCE MASS SPECTROGRAPHIC ANALYSIS

IAD No.: 97-E697-116-28

CONCENTRATION IN $\mu\text{g/ml}$

ELEMENT	CONC.	ELEMENT	CONC.	ELEMENT	CONC.	ELEMENT	CONC.
Uranium		Terbium		Ruthenium		Vanadium	0.001
Thorium		Gadolinium		Molybdenum	0.008	Titanium	0.3
Bismuth		Europium		Niobium		Scandium	<0.001
Lead	0.04	Samarium		Zirconium	0.002	Calcium	1
Thallium		Neodymium		Yttrium		Potassium	3
Mercury	NR	Praseodymium		Strontium	0.002	Chlorine	0.04
Gold		Cerium	<0.001	Rubidium	<0.001	Sulfur	MC
Platinum		Lanthanum		Bromine	0.004	Phosphorus	3
Iridium		Barium	0.05	Selenium	0.006	Silicon	1
Osmium		Cesium	<0.001	Arsenic		Aluminum	>0.7
Rhenium		Iodine		Germanium		Magnesium	0.6
Tungsten		Tellurium	0.01	Gallium	0.002	Sodium	MC
Tantalum	≤ 0.002	Antimony		Zinc	0.2	Fluorine	≈ 0.3
Hafnium		Tin	0.05	Copper	0.2	Oxygen	NR
Lutetium		Indium	STD	Nickel	0.2	Nitrogen	NR
Ytterbium		Cadmium		Cobalt	0.004	Carbon	NR
Thulium		Silver	0.03	Iron	0.4	Boron	0.003
Erbium		Palladium		Manganese	0.04	Beryllium	
Holmium		Rhodium		Chromium	0.08	Lithium	0.005
Dysprosium						Hydrogen	NR

STD - Internal Standard

NR - Not Reported

All elements not detected < 0.001 $\mu\text{g/ml}$

MC - Major Component > 10 $\mu\text{g/ml}$

INT - Interference

Approved:

M. J. Jacobs

COMMERCIAL TESTING & ENGINEERING CO.

GENERAL OFFICES: 938 NORTH LA SALLE STREET, CHICAGO, ILLINOIS 60601 • AREA CODE 312 736-9434
INSTRUMENTAL ANALYSIS DIVISION, 14235 WEST 44TH AVENUE, GOLDEN, COLORADO 80401, PHONE: 303-378-9521

Reply to

To: Mr. Brent Higginbotham
Acurex Corporation
485 Clyde Avenue
Mountain View, California 94042



Date: September 26, 1980

Analyst: T. Bouts

P. O. No.:

Sample No.: 1369

SPARK SOURCE MASS SPECTROGRAPHIC ANALYSIS

IAD No.: 97-E697-116-28

MAN TEST

CONCENTRATION IN $\mu\text{g/ml}$

TANK WATER SAMPLE		AFT. TEST					
ELEMENT	CONC.	ELEMENT	CONC.	ELEMENT	CONC.	ELEMENT	CONC.
Uranium		Terbium		Ruthenium		Vanadium	0.002
Thorium		Gadolinium		Molybdenum	0.2	Titanium	0.2
Bismuth		Europium		Niobium		Scandium	0.01
Lead	0.09	Samarium		Zirconium	0.03	Calcium	MC
Thallium		Neodymium		Yttrium	≤ 0.2	Potassium	3
Mercury	NR	Praseodymium		Strontium	0.009	Chlorine	* MC
Gold		Cerium		Rubidium		Sulfur	MC
Platinum		Lanthanum		Bromine	0.1	Phosphorus	0.8
Iridium		Barium	0.02	Selenium	0.1	Silicon	6
Osmium		Cesium		Arsenic	≤ 0.009	Aluminum	0.7
Rhenium		Iodine	0.004	Germanium		Magnesium	2
Tungsten		Tellurium	0.006	Gallium	0.002	Sodium	2
Tantalum	≤ 0.04	Antimony	0.02	Zinc	MC	Fluorine	=0.2
Hafnium		Tin	≤ 0.006	Copper	MC	Oxygen	NR
Lutetium		Indium	STD	Nickel	* 0.7	Nitrogen	NR
Ytterbium		Cadmium	0.006	Cobalt	* 0.07	Carbon	NR
Thulium		Silver		Iron	7	Boron	* 0.03
Erbium		Palladium		Manganese	0.1	Beryllium	
Holmium		Rhodium		Chromium	0.5	Lithium	0.003
Dysprosium						Hydrogen	NR

STD - Internal Standard

NR - Not Reported

All elements not detected $< 0.002 \mu\text{g/ml}$

MC - Major Component $> 10 \mu\text{g/ml}$

INT - Interference

Approved:

M. J. Roberts
* Heterogeneous

COMMERCIAL TESTING & ENGINEERING CO.

GENERAL OFFICES: 339 NORTH LA SALLE STREET, CHICAGO, ILLINOIS 60601 - AREA CODE 312 739-8434
INSTRUMENTAL ANALYSIS DIVISION, 14235 WEST 44TH AVENUE, GOLDEN, COLORADO 80401, PHONE: 303-278-7521

Reply to

To: Mr. Brent Higginbotham
Acurex Corporation
485 Clyde Ave.
Mountain View, CA 94042



Date: September 23, 1980

Analyst: T. Bouts

P. O. No.:

Sample No.: 80-1473

XAD - MAN TEST

SPARK SOURCE MASS SPECTROGRAPHIC ANALYSIS
CONCENTRATION IN PPM WEIGHT

IAD No.: 97-E697-116-28

ELEMENT	CONC.	ELEMENT	CONC.	ELEMENT	CONC.	ELEMENT	CONC.
Uranium		Terbium		Ruthenium		Vanadium	<0.1
Thorium		Gadolinium		Molybdenum	1	Titanium	*2
Bismuth		Europium		Niobium		Scandium	*<0.1
Lead	0.3	Samarium		Zirconium	<0.1	Calcium	62
Thallium		Neodymium		Yttrium		Potassium	22
Mercury	NR	Praseodymium		Strontium	0.2	Chlorine	1
Gold		Cerium	0.3	Rubidium		Sulfur	14
Platinum		Lanthanum	0.4	Bromine	0.8	Phosphorus	15
Iridium		Barium	0.9	Selenium	<0.1	Silicon	*500
Osmium		Cesium		Arsenic	<0.1	Aluminum	5
Rhenium		Iodine		Germanium		Magnesium	8
Tungsten		Tellurium		Gallium	*<0.1	Sodium	22
Tantalum	0.7	Antimony		Zinc	6	Fluorine	*=0.3
Hafnium		Tin		Copper	6	Oxygen	NR
Lutetium		Indium	STD	Nickel	180	Nitrogen	NR
Ytterbium		Cadmium		Cobalt	0.7	Carbon	NR
Thulium		Silver		Iron	26	Boron	<0.1
Erbium		Palladium		Manganese	1	Beryllium	
Holmium		Rhodium		Chromium	11	Lithium	<0.1
Dysprosium						Hydrogen	NR

* Heterogeneous

STD - Internal Standard

NR - Not Reported

All elements not detected < 0.1 ppm

MAC - Major Component

INT - Interference

Approved:

M. J. Specks

COMMERCIAL TESTING & ENGINEERING CO.

GENERAL OFFICES: 898 NORTH LA SALLE STREET, CHICAGO, ILLINOIS 60601 • AREA CODE 312 786-6434
INSTRUMENTAL ANALYSIS DIVISION, 14335 WEST 64TH AVENUE, GOLDEN, COLORADO 80401, PHONE 303-278-9531

Reply to

To: Mr. Brent Higginbotham
Acurex Corporation
485 Clyde Ave.
Mountain View, CA 94042



Date: September 22, 1980

Analyst: L. Jacobs

P. O. No.:

Sample No.: 80-1562

SPARK SOURCE MASS SPECTROGRAPHIC ANALYSIS
CONCENTRATION IN PPM WEIGHT

IAD No.: 97-E697-116-28

DIESEL OIL - MAN TEST

ELEMENT	CONC.	ELEMENT	CONC.	ELEMENT	CONC.	ELEMENT	CONC.
Uranium		Terbium		Ruthenium		Vanadium	0.02
Thorium		Gadolinium		Molybdenum	0.1	Titanium	5
Bismuth		Europium		Niobium		Scandium	<0.01
Lead	0.2	Samarium		Zirconium	0.04	Calcium	MC
Thallium		Neodymium		Yttrium	0.02	Potassium	7
Mercury		Praseodymium		Strontium		Chlorine	*70
Gold		Cerium		Rubidium	<0.01	Sulfur	0.2
Platinum		Lanthanum		Bromine	0.3	Phosphorus	1
Iridium		Barium	0.3	Selenium		Silicon	25
Osmium		Cesium		Arsenic	0.02	Aluminum	MC
Rhenium		Iodine		Germanium		Magnesium	2
Tungsten		Tellurium	0.2	Gallium	0.02	Sodium	4
Tantalum		Antimony		Zinc	0.7	Fluorine	=1
Hafnium		Tin	0.03	Copper	0.5	Oxygen	NR
Lutetium		Indium	STD	Nickel	1	Nitrogen	NR
Ytterbium		Cadmium	0.02	Cobalt	0.04	Carbon	NR
Thulium		Silver		Iron	10	Boron	0.04
Erbium		Palladium		Manganese	0.2	Beryllium	
Holmium		Rhodium		Chromium	0.4	Lithium	0.02
Dysprosium						Hydrogen	NR

* Heterogeneous

STD - Internal Standard

NR - Not Reported

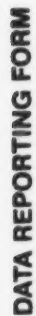
All elements not detected < 0.01 ppm

MC - Major Component > 100 ppm

INT - Interference

Approved:

M. Jacobs



DATA REPORTING FORM

MAN FACE SYSTEM

*All water tank samples were below 1 ug/L

ANALYST G. N. Reed

REVIEWER Erig Neill

Form EED-0637 4-80

COMMERCIAL TESTING & ENGINEERING CO.

GENERAL OFFICES: 228 NORTH LA SALLE STREET, CHICAGO, ILLINOIS 60601 AREA CODE 312 726-8434



Reply to
Instrumental Analysis Division
490 Orchard Street
Golden, CO 80401

Phone: 303-278-9521

November 18, 1980

Mr. Brent Higginbotham
Acurex Aerotherm Corp.
485 Clyde Avenue
Mountain View, CA 94042

RE: IAD #97-E697-116-28

Analytical Report

We were asked as per phone conversation with Carlo Castaldini, on October 20, 1980, to check by atomic absorption, several elemental values on samples that were analyzed by spark source mass spectrometry (SSMS) and reported September 24, 1980.

Nickel was checked on the XAD Resins Acurex ID Nos. 80-1473 & 1533-37. Nickel contamination was due to combustion in the Parr combustion apparatus as they were prepared for SSMS. The samples were reprepared by ashing at approximately 600° C, dissolving the ash in aqua regia, and diluting with deionized water. Nickel was determined from this solution as well by flame atomic absorption.

Copper was found to be a major component on samples 80-1368 and 80-1369 by SSMS. This was quantitated by flame atomic absorption on a diluted portion of each of the samples.

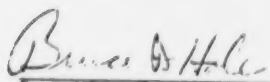
The results of these analyses are presented in Table No. 1 and are reported in the appropriate units on the samples "as received".

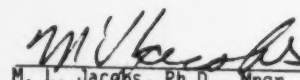


BILLINGS MT • BIRMINGHAM AL • CHARLESTON WV • CLARKSBURG WV • CLEVELAND OH • DENVER CO • GOLDEN CO • HENDERSON KY • JASPER AL • MIDDLEBORO KY
MOBILE AL • NEW BETHLEHEM PA • NEW ORLEANS LA • NORFOLK VA • PALM BEACH FL • PINEVILLE KY • SALINA UT • ST. LOUIS MO • TOLEDO OH • VANCOUVER B.C. CAN

<u>Table No. 1</u>		
<u>Sample #</u>	<u>Nickel</u> <u>(ug/g)</u>	<u>Copper</u> <u>(mg/l)</u>
80-1473 MAN XAD	2.4	---
80-1368 Tap water prior to test---		505
80-1369 Tank water after test ---		480

If there are any questions concerning these results, please call.


 Bruce A. Hale
 Section Supervisor


 M. L. Jacobs, Ph.D., Mngr.
 Instrumental Analysis Div.

85

COMMERCIAL TESTING & ENGINEERING CO.

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 For Your Protection
 P-466



IC ANALYSIS SHEET

Contractor Acurex F,Cl,Br,NO₂,NO₃

Sample Site Mountain View Sample Acquisition Date May 21, 1980

Type of Source _____

Test Number _____ Sample ID Number _____

Sample Description Water Tank

Analyst Responsible E. Hagmann Date Analyzed July 21, 1980 Time 8pm - 2am

Calculations and Report Reviewed By G. Nicoll Report Date July 23, 1980

Instrument Micromeritics - Wescan Conductivity

Eluent 0.0025M Sodium Benzoate @ pH 6.0

Column Flow Rate 1.50 ml/min Pressure 1.08 KPSI Recorder Speed 0.5 cm/min

Sample Size 100 ul Attenuator Setting 2

Original Sample Volume or Mass _____ Multiple Standard Addition: Yes _____ No X

Observations 30 min run, good separation

IC ANALYSIS SHEET

SO₃, SO₄

Contractor Acurex

Sample Site Mountain View Sample Acquisition Date May 21, 1980

Type of Source _____

Test Number _____ Sample IC Number _____

Sample Description Water Tank

Analyst Responsible E. Hagmann Date Analyzed July 11, 1980 Time 8am - 8pm

Calculations and Report Reviewed By G. Nicoll Report Date July 23, 1980

Instrument Micromeritics - Wescan Conductivity

Eluent 0.0047M KHP @ pH 4.5

Column Flow Rate 2.00 ml/min Pressure 0.79 KPSI Recorder Speed 1 cm/min

Sample Size 10 ul Attenuator Setting 4

Original Sample Volume or Mass _____ Multiple Standard Addition: Yes _____ No X

Observations Spike and recovery: average recovery at 88%

IC ANALYSIS SHEET

PO₄

Contractor Acurex

Sample Site Mountain View Sample Acquisition Date May 21, 1980

Type of Source _____

Test Number _____ Sample ID Number _____

Sample Description Water Tank

Analyst Responsible E. Hagmann Date Analyzed July 22, 1980 Time 8am - 3pm

Calculations and Report Reviewed By G. Nicoll Report Date July 23, 1980

Instrument Micromeritics - Wescan Conductivity

Eluent 5 x 10⁻³ M Phthalic Acid

Column Flow Rate 2.00 ml/min Pressure 1.43 KPSI Recorder Speed 0.5 cm/min

Sample Size 100 u1 Attenuator Setting 2

Original Sample Volume or Mass _____ Multiple Standard Addition: Yes _____ No X

Observations Spike and recovery: 106% recovery

Water Tank Blank- Tap Water

Ion	Uncorrected Sample Value	Blank Value	Corrected Sample Value	High/Low Calibration Standards or Concentration Added	Dilution Factor	Assigned Concentration*	Detection Limit*
F ⁻	ND	ND		5, 50		ND	1
Cl ⁻	2	ND	2	5, 50		2	1
Br ⁻	ND	ND		5, 50		ND	5
NO ₂ ⁻	ND	ND		5, 50		ND	2
NO ₃ ⁻	ND	ND		5, 50		ND	5
SO ₃ ⁼	ND	ND		50		ND	10
SO ₄ ⁼	ND	ND		50, 500, 1000		ND	10
PO ₄ ⁼	ND	ND		10, 50		ND	3

*Results: Mg/L values (in original sample or 1 - Interference; MC - major constituent, not quantified; NC - not computed; NG - sample value below blank; ND - not detectable (< 2 σ blank or baseline).

Water Tank- Sample Blank

Ion	Uncorrected Sample Value	Blank Value	Corrected Sample Value	High/Low Calibration Standards or Concentration Added	Dilution Factor	Assigned Concentration*	Detection Limit*
F ⁻	ND	ND		5, 50		ND	1
Cl ⁻	2	ND	2	5, 50		2	1
Br ⁻	ND	ND		5, 50		ND	5
NO ₂ ⁻	ND	ND		5, 50		ND	2
NO ₃ ⁻	ND	ND		5, 50		ND	5
SO ₃ ⁼	ND	ND		50		ND	10
SO ₄ ⁼	ND	ND		50, 500, 1000		ND	10
PO ₄ ⁼	ND	ND		10, 50		ND	3

*Results: mg/L values (in original sample or 1 - Interference; MC - major constituent, not quantified; NC - not computed; NG - sample value below blank; ND - not detectable (< 2 σ blank or baseline).

Water Tank - Prior to Test

Ion	Uncorrected Sample Value	Blank Value	Corrected Sample Value	High/Low Calibration Standards or Concentration Added	Dilution Factor	Assigned Concentration*	Detection Limit*
F ⁻	ND	ND		5, 50		ND	3
Cl ⁻	3	ND	3	5, 50		3	2
Br ⁻	ND	ND		5, 50		ND	10
NO ₂ ⁻	ND	ND		5, 50		ND	10
NO ₃ ⁻	7	ND	7	5, 50		7	5
SO ₃ ⁼	ND	ND		50		ND	10
SO ₄ ⁼	990	ND	990	50, 500, 1000		990	10
PO ₄ ⁼	ND	ND		10, 50		ND	20

*Results: mg/L values (in original sample or 1 - Interference; MC - major constituent, not quantified; NC - not computed; NG - sample value below blank; ND - not detectable (< 2 σ blank or baseline).

Water Tank - Sample *End of test*

Ion	Uncorrected Sample Value	Blank Value	Corrected Sample Value	High/Low Calibration Standards or Concentration Added	Dilution Factor	Assigned Concentration*	Detection Limit*
F ⁻	ND	ND		5, 50		ND	3
Cl ⁻	3	ND	3	5, 50		ND	2
Br ⁻	ND	ND		5, 50		ND	10
NO ₂ ⁻	ND	ND		5, 50		ND	10
NO ₃ ⁻	7	ND	7	5, 50		7	5
SO ₃ ⁼	ND	ND		50		ND	10
SO ₄ ⁼	1000	ND	1000 gr	50, 500, 1000		1000	10
PO ₄ ⁼	ND	ND		10, 50		ND	20

*Results: mg/L values (in original sample or 1 - Interference; MC - major constituent, not quantified; NC - not computed; NG - sample value below blank; ND - not detectable (<2 σ blank or baseline).

5.5 ORGANIC ANALYSIS REPORT

ORGANIC COMPOUNDS (bp < 100° C)

Contractor Acurex
 Sample Site Mountain View Sample Acquisition Date May 21, 1980 Time 14:05
 Type of Source Residential Hot Water Heating System
 Test Number No 1 Sample ID Number End of burn cycle
 Sample Description Exhaust gas
 Analyst Responsible M. Gaudin Date Analyzed May 23, 1980 Time _____
 Calculations and Report Reviewed By G. Nicoll Report Date May 23, 1980

Workup

1. Column Flow Rate (mL/min) _____ 2. Recorder Speed 1 cm/min
 3. Full Scale (mV) 1.0 4. Column Pressure (psi) _____
 5. Electrometer Set (A/mV) 10⁻¹² 6. Calibration Date May 21, 1980
 7. Sample Size (mL) 1.0 8. Oven Temperature (°C) 150
 9. Flame Flow Rates (mL/min): H₂ _____ Air _____
 10. Attenuation 8 11. Range _____
 12. Observations Integrator at 12,000 cft/min

Results: PPM value (in original sample) or I - interference; NC - not computed; NG - sample value below blank;
 ND - not detectable (< 2 σ blank or baseline).

	Uncorrected Sum of Peak Areas	Blank Value	Retention Time	Corrected Sum of Peak Areas	Conc. (%)	PPM/σH ² Sensitivity	High/Low Calibration Standards	Conc. (ppm)
H ₁	1.0	ND	0.7 min	1.0		0.087	9.9 ppm	< 0.2
H ₂	ND	ND	1.0 min	ND		0.11	10.5 ppm	< 0.2
H ₃	ND	ND	1.7 min	ND		0.17	11.2 ppm	< 0.5
H ₄	ND	ND	3.9 min	ND		0.28	11.3 ppm	< 0.5
H ₅	ND	ND	9.4 min	ND		0.37	12 ppm	< 1.0
H ₆	ND	ND	23.2 min	ND		0.53	11.1 ppm	< 1.0
H ₇	NC							

ORGANIC COMPOUNDS (bp < 100° C)

Contractor ALCANTARA
 Sample Site MOUNTAIN VIEW Sample Acquisition Date May 21, 1980 Time 12:45
 Type of Source Residential Hot Water Heating System
 Test Number No. 1 Sample ID Number 9 min into burn cycle
 Sample Description exhaust gas
 Analyst Responsible H. Gardner Date Analyzed May 23, 1980 Time _____
 Calculations and Report Reviewed By C. Nicoll Report Date May 23, 1980

Workup

1. Column Flow Rate (mL/min) _____ 2. Recorder Speed 1 cm/min
 3. Full Scale (mV) 1.0 4. Column Pressure (psi) _____
 5. Electrode Set (A/mV) 10⁻¹² 6. Calibration Date May 21, 1980
 7. Sample Size (mL) 1.0 8. Oven Temperature (°C) 150
 9. Flame Flow Rates (mL/min): H₂ _____ Air _____
 10. Attenuation 8 11. Range _____
 12. Observations Integrator at 12,000 ft²/min

Results: PPM value (in original sample) or I - interference; NC - not computed; ND - sample value below blank;
 ND - not detectable (< 2 σ blank or baseline).

	Uncorrected Sum of Peak Areas	Blank Value	Retention Time	Corrected Sum of Peak Areas	Conc. (%)	PPM/act Sensitivity	High/Low Calibration Standards	Conc. (ppm)
U ₁	1.	ND	0.7 min	1.0		0.087	9.9 ppm	< 0.2
U ₂	ND	ND	1.0 min	ND		0.11	12.5 ppm	< 0.2
U ₃	ND	ND	1.7 min	ND		0.17	11.2 ppm	< 0.5
U ₄	ND	ND	3.3 min	ND		0.28	11.3 ppm	< 0.5
U ₅	ND	ND	9.4 min	ND		0.37	12 ppm	< 1.
U ₆	ND	ND	23.8 min	ND		0.53	11.1 ppm	< 1.
U ₇	NC							

ORGANIC COMPOUNDS (bp < 100° C)

Contractor Acurex
 Sample Site Mountain View Sample Acquisition Date May 21, 1980 Time 15:10
 Type of Source Residential Hot Water Heating System
 Test Number No. 1 Sample ID Number Beginning of burn cycle
 Sample Description Exhaust gas
 Analyst Responsible M. Gardner Date Analyzed May 23, 1980 Time _____
 Calculations and Report Reviewed By G. Nicoll Report Date May 23, 1980

Workup

1. Column Flow Rate (mL/min) _____ 2. Recorder Speed 1 cm/min
 3. Full Scale (mV) 1.0 4. Column Pressure (psi) _____
 5. Electrometer Set (A/mV) 10⁻¹² 6. Calibration Date May 21, 1980
 7. Sample Size (mL) 1.0 8. Oven Temperature (°C) 150
 9. Flame Flow Rates (mL/min): H₂ _____ Air _____
 10. Attenuation 8 11. Range _____
 12. Observations Integrator at 12,000 ft³/min

Results: PPM values (in original sample) or I - interference; NC - not computed; NG - sample value below blank;
 ND - not detectable (< 2 σ blank or baseline).

	Uncorrected Sum of Peak Areas	Blank Value	Retention Time	Corrected Sum of Peak Areas	Conc. (%)	PPM/Std Sensitivity	High/Low Calibration Standards	Conc. (ppm)
16 ₁	5	ND	0.7 min	5		0.087	9.9 ppm	0.4
16 ₂	ND	ND	1.0 min	ND		0.11	12.5 ppm	<0.2
16 ₃	ND	ND	1.7 min	ND		0.17	11.2 ppm	<0.5
16 ₄	ND	ND	3.9 min	ND		0.28	11.3 ppm	<0.5
16 ₅	ND	ND	9.4 min	ND		0.37	12.0 ppm	<1.0
16 ₆	ND	ND	23.8 min	ND		0.53	11.1 ppm	<1.0
16 ₇	NG							



DATA REPORTING FORM

CUSTOMER _____ CMEA _____ DATE July 15, 1980
CUSTOMER CONTRACT NO. 307605.22 ACUREX CONTRACT NO. _____
RESULTS REPORT TO _____ TELEPHONE _____
ADDRESS _____

ADDRESS
Mountain View May 21, 1980
D. J. Dine M. W. Ferguson

[illegible]

M. Gardner, G. Nicoll

REVIEWER

Form EEO-057 4-80

Thia Need!

SAMPLE:

Filter Mountain View 5/21/80

[illegible]

SAMPLE: Filter Blank

SAMPLE: Filter Blank

[illegible]

SAMPLE:

XAD Mountain View 5/21/80

[illegible]

SAMPLE: XAD Blank

SAMPLE: XAD Blank

No Peaks

SAMPLE: OMC Mountain View 5/21/80

SAMPLE: OMC Mountain View 5/21/80

97

IN REPORT

SAMPLE: Tap Water Mountain View 5/21/80

[illegible]

SAMPLE: Tank Blank Mountain View 4/21/80

SAMPLE: Tank Blank Mountain View 4/21/80

[illegible]

SAMPLE: Tank Sample Mountain View 5/21/80

SAMPLE:

Tank Sample Mountain View 5/21/80

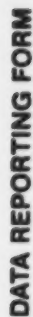
[illegible]

COMPOUNDS SOUGHT
Detection Limit in nanograms

8	* 4-bromophenyl phenyl ether	40	* indeno(1,2,3-cd)pyrene
1	* bis (2-chloroisopropyl) ether	2	* pyrene
2	* bis (2-chloroethoxy) methane	20	2,3,7,8-tetrachlorodibenzo-p-dioxin
8	* hexachlorobutadiene	2	* acenaphthene
40	* hexachlorocyclopentadiene	100	* benzidine
1	* isophorone	8	* 1,2,4-trichlorobenzene
1	* naphthalene	8	* hexachlorobenzene
8	* nitrobenzene	8	* hexachloroethane
4	* N-nitrosodiphenylamine	3	* bis(2-chloroethyl)ether
40	* N-nitrosodi-n-propylamine	2	* 2-chloronaphthalene
3	* bis (2-ethylhexyl) phthalate	4	* 1,2-dichlorobenzene
3	* butyl benzyl phthalate	8	* 1,3-dichlorobenzene
1	* di-n-butyl phthalate	4	* 1,4-dichlorobenzene
2	* di-n-octyl phthalate	20	* 3,3-dichlorobenzindine
2	* diethyl phthalate	10	* 2,4-dinitrotoluene
2	* dimethyl phthalate	10	* 2,6-dinitrotoluene
5	* benzo(a)anthracene	1	* 1,2-diphenylhydrazine (as azobenzene)
7	* benzo(a)pyrene	2	* fluoranthene
8	* 3,4-benzofluoranthene	4	* 4-chlorophenyl phenyl ether
8	* benzo(k)fluoranthene	40	anthanthrene
5	* chrysene	40	benzo(e)pyrene
1	* acenaphthylene	@	dibenzo(a,h)pyrene
1	* anthracene	@	dibenzo(a,i)pyrene
40	* benzo(ghi)perylene	40	dibenzo(c,g)carbazole
2	* fluorene	40	7,12 dimethyl benz(a)anthracene
1	* phenanthrene	40	3-methyl cholanthrene
40	* dibenzo(a,h)anthracene	40	perylene
		40	Benzo(c)phenanthrene

*Authentic standard ran

@Molecular weight too high for direct analysis by Base/Neutral run



July 21, 1980

CUSTOMER _____
CHEA _____
DATE July 21, 1980

CUSTOMER CONTRACT NO. 307605.22 ACUREX CONTRACT NO.

RESULTS REPORT TO _____ TELEPHONE _____

ADDRESS	
Mountain View	28.5 dscm

*Sample Aliquot All water tank blanks are clean

ANALYST

Form EEO-0517 4-80

REVIEWER

SAMPLE: Man XAD #1473

	TCO mg	GRAV mg	TCO + GRAV Total mg	Concentration mg/dscm
Total Sample ¹	74	18	92	3.2
Taken for LC ²	36	9	45	1.6
Recovered ³	26	14	40	1.4

Fraction	TCO in mg				GRAV in mg				TCO + GRAV Total mg	Concentration mg/dscm
	Found in Fraction	Blank	Cor- rected	Total ⁴	Found in Fraction	Blank	Cor- rected	Total ⁴		
1	25	<0.02	25	52	11	<1	11	23	75	2.6
2	0.09	<0.01	0.09	0.2	1.0	<0.8	1	2	2	0.07
3	0.41	<0.01	0.41	0.85	<0.8	<0.8	<0.8	<2	<3	0.1
4	0.06	<0.01	0.06	0.1	1.2	1.2	<0.8	<2	<2	<0.07
5	<0.01	<0.01	<0.01	<0.02	<0.8	<0.8	<0.8	<2	<2	<0.07
6	<0.01	<0.01	<0.01	<0.02	1.0	<0.8	1.0	2	2	0.07
7	<0.01	<0.01	<0.01	<0.02	2.0	<0.8	2.0	4	4	0.1
Sum	26	<0.08	26	54	16	<6	15	31	85	3.0

1. Quantity in entire sample, determined before LC
2. Portion of whole sample used for LC, actual mg
3. Quantity recovered from LC system, actual mg
4. Total mg computed back to total sample



SAMPLE: MAN XAD 1473 F1

SAMPLE: MAN XAD 1473 F1

[illegible]

SAMPLE:

MAN XAD 1473 F2

No Peaks

SAMPLE: MAN XAD 1473 F3

SAMPLE: MAN XAD 1473 F3

[illegible]

SAMPLE:

SAMPLE: MAN XAD 1473 F4

[illegible]

SAMPLE:

MAN XAD 1473 F5

[illegible]

IN REPORT
SAMPLE: MAN XAD 1473 F6

SAMPLE: MAN XAD 1473 F6

[illegible]

SAMPLE:

MAN XAD 1473 F7

[illegible]

SAMPLE: 1473 F1 Mountain View XAD

Intensity	Category	MW Range
1	Aliphatic hydrocarbons	

[illegible]

5.6 BIOASSAY ANALYSES REPORT

GENETICS ASSAY NO. 5397

LBI SAFETY NO. 6391

CYTOTOXIC EVALUATION OF

SAMPLE #1473
(CMEA MOUNTAIN VIEW)

IN THE
RODENT CELL (CHO)
CLONAL TOXICITY ASSAY

FINAL REPORT

SUBMITTED TO:

ACUREX CORPORATION
485 CLYDE AVENUE
MOUNTAIN VIEW, CALIFORNIA 94042

SUBMITTED BY:

LITTON BIONETICS, INC.
5516 NICHOLSON LANE
KENSINGTON, MARYLAND 20795

LBI PROJECT NO. 20933

REPORT DATE: DECEMBER, 1980

PREFACE

This report contains a summary of the data compiled during the evaluation of the test compound. The report is organized to present the results in a concise and easily interpretable manner. The first part contains items I - IX. Items I - IV provide sponsor and compound identification information, type of assay, and the assay design reference number. All assay design references indicate a standard procedure described in the Litton Bionetics, Inc. "Screening Program for the Identification of Potential Mutagens and Carcinogens." Item V provides the initiation and completion dates for the study, and Item VI provides identification of supervisory personnel. Item VII identifies the tables and figures containing the data used by the study director in interpreting the test results. The interpretation itself is in Item VIII. Item IX provides the conclusion and evaluation.

The second part of the report, entitled Assay Design, describes the materials and procedures employed in conducting the assay. This part of the report also contains any appendices, as well as evaluation criteria used by the study director. The evaluation criteria are included to acquaint the sponsor with the methods used to develop and analyze the test results.

All test and control results presented in this report are supported by fully documented raw data which are permanently maintained in the files of the Department of Genetics and Cell Biology or in the archives of Litton Bionetics, Inc., 5516 Nicholson Lane, Kensington, Maryland, 20795.

Copies of raw data will be supplied to the sponsor upon request.

- I. SPONSOR: Acurex Corporation
- II. MATERIAL (TEST COMPOUND): GENETICS ASSAY NUMBER: 5397
- A. Identification: Sample #1473 (CMEA Mountain View)
- B. Date Received: October 9, 1980
- C. Physical Description: Brown, opaque liquid
- III. TYPE OF ASSAY: Rodent Cell (CHO) Clonal Toxicity Assay
- IV. ASSAY DESIGN NUMBER: 442
- V. STUDY DATES:
- A. Initiation: October 20, 1980
- B. Completion: November 24, 1980
- VI. SUPERVISORY PERSONNEL:
- A. Study Director: Brian C. Myhr, Ph.D.
- B. Laboratory Supervisor: Robert Young, M.S.
- VII. RESULTS:
- The data are presented in Table 1 on page 4 and in Figure 1 on page 5.
- VIII. INTERPRETATION OF RESULTS:
- The test material, Sample No. 1473, was supplied as a SASS train organic extract in 10 ml of methylene chloride. The total organic content was given as 26 mg. A portion of the sample (8.20 ml) was solvent exchanged into an equal volume of dimethylsulfoxide (DMSO). The concentration of organics was therefore unchanged, and the value of 2.6 mg/ml (or 2.6 $\mu\text{g}/\mu\text{l}$) was used to convert the doses obtained on a volume basis into the equivalent amount of organics per milliliter of culture medium.
- The test material remained completely soluble after exchange into DMSO. Diluted stocks were prepared with DMSO just prior to testing, and the treatments were initiated with culture media containing 1:100 dilutions of the stocks. The highest test concentration was achieved with a 1:50 dilution of the solvent-exchanged sample, which introduced 2% DMSO into the medium. Vehicle control cultures were therefore exposed to

VIII. INTERPRETATION OF RESULTS: (continued)

culture media containing 1% and 2% DMSO in order to provide the reference points for determining the survival of the cells to treatments with the test material. No precipitation of test material in the culture medium was observed at any of the tested concentrations.

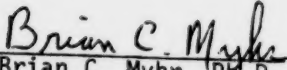
Seven test concentrations from 0.05 μ l/ml to 20 μ l/ml were evaluated for their effects on colony survival. As shown in Table 1 and Figure 1, none of the assayed concentrations caused significant changes in the numbers of colonies. This lack of observable toxicity was not conclusive, however, because the highest concentration corresponded to only 52 μ g of organics/ml, which was essentially the midpoint of the concentration range where an EC50 would yield a moderate toxicity classification (see Evaluation Criteria). A sharp increase in toxicity could occur with a small increase in concentration, but it is reasonable to assume that 50% survival would not have been observed had the high dose been doubled. This concentration would have fallen just within the low toxicity region. Thus, the test material was evaluated as possibly having low toxicity or no detectable toxicity to CHO cells. The position of the EC50 could be further investigated by concentrating the extracted organic material.

IX. CONCLUSIONS:

The test material, Sample No. 1473, was evaluated as having low or nondetectable toxicity in the CHO Clonal Toxicity Assay. No toxicity was observed for concentrations up to the highest testable level of 52 μ g of organics/ml.

Submitted by:

Study Director


Brian C. Myhr, Ph.D.
Section Chief
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12/1/80
Date

Reviewed by:


David J. Brusick, Ph.D.
Director
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12/1/80
Date

TABLE 1
RODENT CELL (CHO) CLONAL TOXICITY ASSAY

Sample Identity: <u>Sample #1473, CMEA</u>	EC50 Value: <u>>20 μl/ml (>52 μg/ml)</u>
<u>MOUNTAIN VIEW</u>	Toxicity
Description of Test Sample: <u>Clear, pale</u>	Classification: <u>Probably Low or Non-</u>
<u>yellow solution in DMSO.</u>	<u>detectable</u>
	pH Alterations: <u>NONE</u>
LBI Assay No.: <u>5397</u>	Comments on Treatment: _____
Date Received: <u>October 9, 1980</u>	_____
Test Date: <u>November 17, 1980</u>	_____
Vehicle: <u>DMSO</u>	_____
Cell Type: <u>CHO-K1</u>	_____
Cells Seeded per Dish: <u>200</u>	_____

CLONAL TOXICITY DATA

Sample	Applied Concentration μ l/ml	Dish #1	Dish #2	Dish #3	Average Count	Relative Survival* %	Cloning Efficiency %
NC	---	207	185	192	194.7	---	97.4
VC, 1%	10.0	180	177	172	176.3	100.0	88.2
VC, 2%	20.0	173	162	166	167.0	100.0	83.5
Test	0.05	183	192	189	188.0	106.6	
Test	0.2	C	192	183	187.5	106.4	
Test	0.5	185	189	179	184.3	104.5	
Test	2.0	161	187	183	177.0	100.4	
Test	5.0	161	190	164	171.7	97.4	
Test	10.0	166	178	180	174.7	99.1	
Test	20.0	170	171	154	165.0	98.8	

NC = Negative Control, F12 medium.

VC = Vehicle Control, percent DMSO in F12 as noted.

* Relative to 2% VC for 20 μ l/ml and to 1% VC for other treatments.

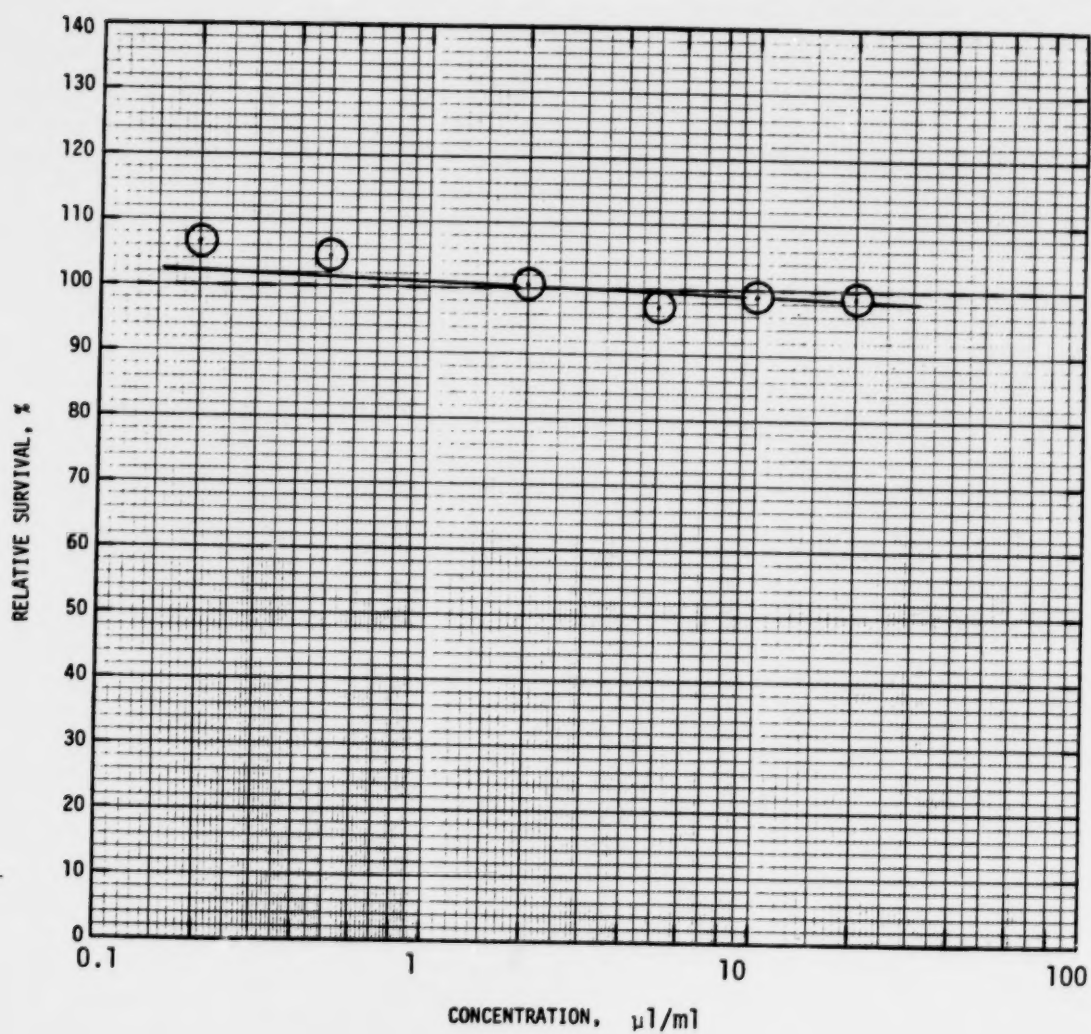
C = Contaminated.

FIGURE 1

RODENT CELL (CHO) CLONAL TOXICITY ASSAY

EC₅₀ DETERMINATION

SAMPLE #1473, CMEA MOUNTAIN VIEW



ASSAY DESIGN NO. 442

RODENT CELL CLONAL (CHO) TOXICITY ASSAY

This assay conforms to one of the bioassay tests in the US Environmental Protection Agency (EPA) IERL - RTP Procedures Manual: Level 1 Environmental Assessment Biological Tests (September, 1980). Level 1 bioassays obtain preliminary information on the harmful biological effects of chemicals found in industrial feed and waste streams by using mammalian cells in culture to measure metabolic impairment and cellular death.

1. OBJECTIVE

The objective of this assay is to determine the concentration of a test article that will reduce the colony forming ability of Chinese hamster cells by 50% after a 24-hour exposure. This concentration is referred to as the EC₅₀ value.

2. MATERIALS

A. Indicator Cells

The indicator cells used in this study are Chinese hamster CHO-K1 cells (American Type Culture Collection No. CCL 61). This cell type was derived from ovarian tissue and has spontaneously transformed to a stable, hypodiploid line of rounded, fibroblastic cells with unlimited growth potential. Monolayer cultures have a fast doubling time of 11 to 14 hours, and untreated cells can normally be cloned with an efficiency of 80% or greater. Permanent stocks are maintained in liquid nitrogen and laboratory cultures are maintained by serial subculturing. Laboratory cultures are periodically checked by culturing methods for the absence of mycoplasma contamination. This test system is specified by the IERL- RTP Procedures Manual.

B. Medium

The CHO-K1 cell line has an absolute requirement for proline and therefore must be maintained in a culture medium containing sufficient amounts of this amino acid. Ham's F12 medium, which contains 3×10^{-4} M L-proline, is used, supplemented with 10% fetal bovine serum, 100 units per milliliter of penicillin, 100 µg streptomycin per milliliter, and 0.5 µg amphotericin B (Fungizone) per milliliter.

C. Controls

Untreated cells are cloned to establish the control cloning efficiency. If the test article is dissolved in an organic solvent (usually dimethylsulfoxide), cells exposed to solvent in the medium

2. MATERIALS (Continued)

are cloned to provide the reference cloning efficiency for the effect of the test article. The final concentration of solvent in the growth medium is generally 1% or less. All controls are performed in triplicate.

D. Sample Forms

Solid samples are tested as a solution or are ground to fine particles (less than 5 μm) and tested as a suspension in growth medium. Dry particulate articles, aqueous liquids, suspensions and slurries are added directly to the growth medium and tested as a suspension or solution. Liquids containing less than 0.2% organic solvent are generally tested directly; samples dissolved in organic solvents are solvent exchanged in dimethylsulfoxide (DMSO) before testing. Original sample volumes may be reduced a maximum of 10-fold during solvent exchange, and the concentration factor is used to convert assayed volumes into equivalent original sample volumes. All sample manipulations are performed as described in the IERL-RTP Level 1 Procedures Manual.

3. EXPERIMENTAL DESIGN

A. Dose Selection

Unless the approximate toxicity is already known or the sample size is limiting, the following dose ranges are tested for different sample forms. Dry particulate articles are dissolved or suspended in growth medium and tested at five dose levels from 1000 $\mu\text{g/ml}$ to 10 $\mu\text{g/ml}$. Aqueous samples, suspensions or slurries are tested from 600 $\mu\text{liters/ml}$ to 6 $\mu\text{liters/ml}$ in five dose steps. Samples that are solvent exchanged into DMSO are tested from 20 $\mu\text{liters/ml}$ to 0.2 $\mu\text{liters/ml}$, also in five dose steps. Solvent concentrations are 1% for all dose levels except the maximum applicable dose (MAD) of 20 $\mu\text{l/ml}$, which contains 2% solvent. A second dose study is performed with an appropriate dose range if the EC_{50} has not been properly located in the initial test; EC_{50} values greater than 1,000 $\mu\text{g/ml}$, 600 μliters of aqueous sample/ml or 20 μliters nonaqueous sample/ml are not determined.

B. Clonal Toxicity Assay

Cells from monolayer stock cultures in logarithmic growth phase are trypsinized, counted by hemacytometer and reseeded into a series of 60- or 100-mm culture dishes at 200 cells per dish. The cultures are incubated for 6 to 16 hours at 37°C to allow attachment of the cells and recovery of growth rate.

Test article is then applied (three dishes per dose), and the cultures returned to the incubator. If the test article causes a color change, an additional dish is treated with the high dose and the pH of the

3. EXPERIMENTAL DESIGN (Continued)

medium is determined. The pH is also recorded for the highest dose which results in a slight color change. After a 24-hour exposure period the medium is aspirated and the cells washed with Dulbecco's phosphate buffered saline (PBS; prewarmed to 37°C). The pH of the discarded medium for which initial pH measurements were made is again recorded. Fresh medium is placed on each culture and incubation continued for an additional 6 days to allow colony development. Medium is drained from the cultures and the surviving colonies are washed with PBS, fixed in methanol, and stained with Giemsa. Colony counting is performed by eye; colonies smaller than 50 to 100 cells are not counted.

4. ASSAY ACCEPTANCE CRITERIA

The assay is considered acceptable for evaluation of the test results if the following criteria are met:

- The average cloning efficiency of the CHO-K1 cells in the negative controls is 70% or greater, but not exceeding 115%.
- The distribution of colonies in the treated cultures is generally uniform over the surface of the culture dish.
- The data points for each test concentration critical to the location of the EC_{50} are the averages of at least two treated cultures.
- A sufficient number of test concentrations are available to clearly locate the EC_{50} within a toxicity region as defined under Assay Evaluation Criteria.
- If the EC_{50} value is greater than 1000 $\mu\text{g/ml}$, 600 μliters of aqueous sample/ml, or 20 μliters of nonaqueous sample/ml, the plotted curve does not exceed 110% of the negative control.

5. ASSAY EVALUATION CRITERIA

The screened doses, pH values (if appropriate), colony counts, percent survivals (colony counts relative to control colony counts) and EC_{50} values are provided. The percent survival is plotted as a function of applied concentration and the EC_{50} value determined graphically by fitting a curve by eye through the data points. The EC_{50} is used to rank the test material using the standard evaluation criteria defined in the table below.

Sorbent extracts of known organic content are evaluated as nonaqueous liquids and in terms of their solid contents ($\mu\text{g organics/ml}$), and are ranked using the more sensitive parameter. Where data on the original liquid or gas sample volumes is provided by the Sponsor, the EC_{50} value is calculated in terms of these equivalent volumes per milliliter of culture medium; however, no evaluations are made as yet on this basis.

5. ASSAY EVALUATION CRITERIA (Continued)

Toxicity ^a	Solids (EC ₅₀ in µg/ml)	Aqueous Liquids (EC ₅₀ in µl/ml)	Nonaqueous Liquids ^b (EC ₅₀ in µl/ml)
High	<10	<6	<.2
Moderate	10 to 100	6 to 60	.2-2
Low	100 to 1000	60 to 600	2-20
Not Detectable	>1000	>600	>20

^aEvaluation criteria formulated by Litton Bionetics, Inc. for IERL-RTP Procedures Manual: Level 1 Environmental Assessment Biological Tests.

^bCriteria for nonaqueous liquids are tentative and under evaluation.

6. RECORDS TO BE MAINTAINED

All raw data, protocols, protocol modifications, test article weight and dispensation records and correspondence between LBI and the Sponsor are being maintained in a central file within the Department of Genetics and Cell Biology. These records will be filed under departmental assay number and held up to 2 years following submission of the final report to the Sponsor. After 2 years they will be transferred to the LBI archives for permanent storage.

Q.A. Inspection Statement
(reference 21 CFR 58.35(b)(7))

PROJECT 20993

LBI Assay No. 5397

TYPE of STUDY Codest Cell (CHO) Clonal Toxicity Assay

This final study report was reviewed by the LBI Quality Assurance Unit on December 1, 1980. A report of findings was submitted to the Study Director and to Management on December 1, 1980.

The short-term nature of this study precluded inspection while it was in process. The Quality Assurance Unit inspects an in-process study of this type approximately once per month to assure that no significant problems exist that are likely to affect the integrity of this type of study.

Patrice M. Cuccaro
Auditor, Quality Assurance Unit

GENETICS ASSAY NO. 5397

LBI SAFETY NO. 6391

MUTAGENICITY EVALUATION OF
SAMPLE #1473

IN THE
AMES SALMONELLA/MICROSOME
PLATE TEST
FINAL REPORT

SUBMITTED TO:

ACUREX CORPORATION
485 CLYDE AVENUE
MOUNTAIN VIEW, CALIFORNIA 94042

SUBMITTED BY:

LITTON BIONETICS, INC.
5516 NICHOLSON LANE
KENSINGTON, MARYLAND 20795

LBI PROJECT NO. 20988

REPORT DATE: DECEMBER, 1980

PREFACE

This report contains a summary of the data compiled during the evaluation of the test compound. The report is organized to present the results in a concise and easily interpretable manner. The first part contains items I-IX. Items I-IV provide sponsor and compound identification information, type of assay, and the protocol reference number. All protocol references indicate a standard procedure described in the Litton Bionetics, Inc. "Screening Program for the Identification of Potential Mutagens and Carcinogens." Item V provides the initiation and completion dates for the study, and Item VI provides identification of supervisory personnel. Item VII identifies the tables and/or figures containing the data used by the study director in interpreting the test results. The interpretation itself is in Item VIII. Item IX provides the conclusion and evaluation.

The second part of the report describes the materials and procedures employed in conducting the assay. This part of the report also contains evaluation criteria used by the study director, and any appendices. The evaluation criteria are included to acquaint the sponsor with the methods used to develop and analyze the test results.

All test and control results presented in this report are supported by fully documented raw data which are permanently maintained in the files of the Department of Genetics and Cell Biology or in the archives of Litton Bionetics, Inc., 5516 Nicholson Lane, Kensington, Maryland 20795.

- I. SPONSOR: Acurex Corporation
- II. MATERIAL (TEST COMPOUND): GENETICS ASSAY NUMBER: 5397
 - A. Identification: Sample #1473
 - B. Date Received: October 9, 1980
 - C. Physical Description: Pale yellow liquid
- III. TYPE OF ASSAY: Ames Salmonella/microsome Mutagenesis Assay
- IV. PROTOCOL NUMBER: 401 (EPA-Level I)
- V. STUDY DATES:
 - A. Initiation: November 7, 1980
 - B. Completion: November 13, 1980
- VI. STUDY DIRECTOR: D.R. Jagannath, Ph.D.
- VII. RESULTS:

The results of this assay are presented in Table 1.

VIII. INTERPRETATION OF RESULTS:

The test material, Sample No. 1473, was supplied as a SASS train organic extract in the 10 ml of methylene chloride. The total organic content was given as 26.0 mg. A portion of the sample (8.20 ml) was solvent exchanged into dimethylsulfoxide (DMSO) to a final volume of 8.20 ml. This solvent exchanged sample (test material) was examined for mutagenic activity in the Ames/Salmonella assay in the presence and absence of liver microsomal enzyme preparations from Aroclor-induced rats.

A negative control consisting of the solvent DMSO and specific positive compounds were also assayed concurrently with the test material.

DOSE RANGE:

The dose range employed for the evaluation of this test material was from 10.0 μ l to 200.0 μ l per plate. Based on the organic content of the sample the doses employed would equal to 26.0 μ g to 520.0 μ g per plate. The tests were conducted using two plates per dose level.

VIII. INTERPRETATION OF RESULTS: (continued)

TOXICITY

The test material did not exhibit toxicity with any of the indicator strains used in this assay.

The results of the tests conducted on the test material in the absence of an activation system was positive with the strain TA-98. The lowest dose at which the response was observed was at 100 μ l or 260.0 μ g organics per plate.

The results of the test conducted on the test material in the presence of rat liver activation system were negative.

IX. CONCLUSIONS:

(1) The SASS train organic extract sample #1473 exhibited genetic activity with the strain TA-98 in the nonactivation assays conducted in this evaluation and is considered as mutagenic under these test conditions. (2) Based on the EPA's 'Definition of Toxicity Categories for Health Effects Tests' the mutagenic activity of the sample is classified as 'Moderate' (see Table A). The specific Activity at the Minimum Effective Concentration (which is the number of revertants minus the background revertants divided by μ g organics x 1000) of the sample (260.0 μ g) based on the results from TA-98 (nonactivation) was 92.31 revertants per mg of organics.

The classification of the mutagenic activity (item 2 above) and the Specific Activity of the sample (item 3 above) are given here to compare the potency of various samples belonging to the same class.

Submitted by:

Study Director

D.R. Jagannath 12/3/80
D.R. Jagannath, Ph.D. Date
Section Chief
Submammalian Genetics
Department of Genetics
and Cell Biology

Reviewed by:

for: David J. Brusick 12/3/80
David J. Brusick, Ph.D. Date
Director
Department of Genetics
and Cell Biology

TABLE A

DEFINITION OF TOXICITY CATEGORIES FOR HEALTH EFFECTS ASSAYS*

Assay ^a	Activity Measured ^b	Sample Type ^c	MAD ^d	Units	Range of Concentration or Dosage		Not Detectable (ND)
					High	Moderate	
Ames	MEC (mutagenesis)	S AL, NAL	5 200	mg/plate μl/plate	<0.05 <2	0.05-0.5 2-20	0.5-5 20-200 ND at >5 ND at >200
RAM	EC ₅₀ (lethality)	S AL NAL	1 600 20	mg/ml μl/ml μl/ml	<0.01 <6 <0.2	0.01-0.1 6-60 0.2-2	0.1-1 60-600 ND at >1 ND at >600 ND at >20
CHO	EC ₅₀ (lethality)	S AL NAL	1 600 20	mg/ml μl/ml μl/ml	<0.01 <6 <0.2	0.01-0.1 6-60 0.2-2	0.1-1 60-600 ND at >1 ND at >600 ND at >20
WAT	LD ₅₀ (lethality and toxic signs)	S AL, NAL	5 5	gm/kg ml/kg	<0.05 <0.05	0.05-0.5 0.5-0.5	0.5-5 0.5-5 ND at <5 ND at <5

^aStandard test abbreviations are as follows:

Ames: Ames Salmonella/microsome mutagenesis assay
RAM: Rabbit alveolar macrophage cytotoxicity assay
CHO: Rodent cell clonal toxicity assay
WAT: Acute in vivo test in rodents (whole animal test)

^bStandard abbreviations for measured endpoints are as follows:

MEC: Minimum effective concentration
EC₅₀: Calculated concentration expected to produce effect in 50 percent of population
LD₅₀: Calculated dose expected to kill 50 percent of population

C_S = Solid, AL = Aqueous liquid, NAL = Nonaqueous liquid

^dMAD = Maximum applicable dose

*Evaluation criteria formulated by Litton Bionetics, Inc. for IERL-RTP Procedures Manual: Level I Environmental Assessment Biological Tests.

RESULTS

TABLE 1

A. NAME OR CODE DESIGNATION OF THE TEST COMPOUND: SAMPLE SIXTY

B. SOLVENT: DMF

C. TEST INITIATION DATE: 11/27/80

D. TEST COMPLETION DATE: 11/13/80

E. S-9 LOT#: 09809

NOTE: CONCENTRATIONS ARE GIVEN IN MICROLITERS PER PLATE

TEST	SPECIES	TISSUE	REVERTANTS PER PLATE											
			TA-1535			TA-1537			TA-98			TA-100		
			1	2	3	1	2	3	1	2	3	1	2	3
NONACTIVATION														
SOLVENT CONTROL	---	---	12	11	6	9	22	18	168	193				
POSITIVE CONTROL	---	---	990	1020	191	225	861	727	1383	1274				
TEST COMPOUND														
10-000000 UL	---	---	22	21	10	10	28	21	155	161				
25-000000 UL	---	---	34	24	9	6	30	21	164	155				
50-000000 UL	---	---	17	21	10	3	30	44	173	152				
100-000000 UL	---	---	21	23	10	8	58	50	176	160				
200-000000 UL	---	---	24	28	14	9	63	33	153	166				
ACTIVATION														
SOLVENT CONTROL	RAT	LIVER	9	9	9	9	14	15	190	198				
POSITIVE CONTROL	RAT	LIVER	118	120	179	161	900	1066	1942	1746				
TEST COMPOUND														
10-000000 UL	RAT	LIVER	9	10	16	15	32	21	191	118				
25-000000 UL	RAT	LIVER	4	10	6	9	37	29	152	135				
50-000000 UL	RAT	LIVER	11	10	8	5	33	16	145	152				
100-000000 UL	RAT	LIVER	6	11	3	3	23	29	193	181				
200-000000 UL	RAT	LIVER	19	16	1	5	29	24	223	226				
..														
TA-1535	SODIUM AZIDE								TA-1535	2-ANTHRAMINE	2.5 UC/PLATE			
TA-1537	9-AMINOACRIDINE								TA-1537	2-ANTHRAMINE	2.5 UC/PLATE			
TA-98	2-NITROFLUORENE								TA-98	2-ANTHRAMINE	2.5 UC/PLATE			
TA-100	SODIUM AZIDE								TA-100	2-ANTHRAMINE	7.5 UC/PLATE			
SOLVENT 200 UL/PLATE														

AMES SALMONELLA/MICROSOME PLATE ASSAY

1. OBJECTIVE

The objective of this study was to evaluate a test material for mutagenic activity in a bacterial assay with and without a mammalian S9 activation system.

2. RATIONALE

The *Salmonella typhimurium* strains used at LBI are all histidine auxotrophs by virtue of mutations in the histidine operon. When these histidine-dependent cells are grown in a minimal media petri plate containing a trace of histidine, only those cells that revert to histidine independence (*his*⁺) are able to form colonies. The trace amount of histidine allows all the plated bacteria to undergo a few divisions; this growth is essential for mutagenesis to occur. The *his*⁺ revertants are easily scored as colonies against the slight background growth. The spontaneous mutation frequency of each strain is relatively constant, but when a mutagen is added to the agar the mutation frequency is increased 2- to 100-fold. Cells which grow to form colonies on the minimal media petri plates are therefore assumed to have reverted, either spontaneously or by the action of a test substance to *his*⁺ genotype.

3. MATERIALS

A. Indicator Microorganisms

The *Salmonella typhimurium* strains used in this assay were obtained from Dr. Bruce Ames, University of California at Berkeley.¹⁻⁵ The following 4 strains were used:

Strain Designation	Gene Affected	Additional Mutations			Mutation Type Detected
		Repair	LPS	R Factor	
TA-1535	<u>his</u> G	Δ <u>uvr</u> B	<u>rfa</u>	-	Base-pair substitution
TA-1537	<u>his</u> C	Δ <u>uvr</u> B	<u>rfa</u>	-	Frameshift
TA-98	<u>his</u> D	Δ <u>uvr</u> B	<u>rfa</u>	pKM101	Frameshift
TA-100	<u>his</u> G	Δ <u>uvr</u> B	<u>rfa</u>	pKM101	Base pair substitution

3. MATERIALS (Continued)

The aforementioned strains have, in addition to the mutation in the histidine operon, a mutation (rfa-) that leads to defective lipopolysaccharide coat, a deletion that covers genes involved in the synthesis of vitamin biotin (bio-) and in the repair of ultraviolet (uv) - induced DNA damage (uvrB-). The rfa- mutation makes the strains more permeable to many large molecules. The uvrB- mutation decreases repair of some types of chemically or physically damaged DNA and thereby enhances the strain's sensitivity to some mutagenic agents. The resistant transfer factor plasmid (R factor) pKM101 in TA-98 and TA-100 is believed to cause an increase in error-prone DNA repair that leads to many more mutations for a given dose of most mutagens⁵. In addition, plasmid pKM101 confers resistance to the antibiotic ampicillin, which is a convenient marker to detect the presence of plasmid in the cells.

All indicator strains are kept at 4°C on minimal medium plates supplemented with a trace of biotin and an excess of histidine. The plates with plasmid-carrying strains contain in addition ampicillin (25 µg/ml) to ensure stable maintenance of plasmid pKM101. New stock culture plates are made as often as necessary from frozen master cultures or from single colony reisolates that were checked for their genotypic characteristics (his, rfa, uvrB, bio) and for the presence of plasmid. For each experiment, an inoculum from the stock culture plates is grown overnight at 37°C in nutrient broth (Oxoid CM67).

B. Media

The bacterial strains were cultured in Oxoid Media #2 (nutrient Broth). The selective medium was Vogel Bonner Medium E with 2% glucose⁷. The overlay agar consisted of 0.6% purified agar with 0.5 mM histidine, 0.05 mM biotin and 0.1 M NaCl according to the methods of Ames et. al.⁶

C. Activation System

(1) S9 Homogenate

A 9,000 x g supernatant prepared from Sprague-Dawley adult male rat liver induced by Aroclor 1254 (described by Ames et. al.⁶) was purchased from Bionetics Laboratory Products, Litton Bionetics, Inc. and used in this assay.

(2) S9 Mix

Components	Concentration per Milliliter S9 Mix
NADP (sodium salt)	4 µmoles
D-glucose-6-phosphate	5 µmoles
MgCl ₂	8 µmoles
KCl	33 µmoles
Sodium phosphate buffer pH 7.4	100 µmoles
Organ homogenate from rat liver (S9 fraction)	100 µliters

4. EXPERIMENTAL DESIGN

A. Dosage Selection

All tests are run at a minimum of four concentrations. In the Standard EPA Level I Ames assays, five dose levels of the test material, dissolved in a suitable solvent, are added to the test system. The standard test doses for the extracted material are 10, 25, 50, 100 and 200 μ liters per plate. The solids are tested up to 5 mg per plate and at lower concentrations of 2.5, 1, 0.5, 0.1 and 0.05 mg per plate. The samples are retested over a narrower range of concentrations with strains showing positive results if there is enough sample.

B. Mutagenicity Testing

The procedure used is based on the paper published by Ames et. al.⁶ and is performed as follows:

(1) Nonactivation Assay

To a sterile 13 x 100 mm test tube placed in a 43°C water bath the following is added in order:

- (a) 2.00 ml of 0.6% agar containing 0.05 mM histidine and 0.05 mM biotin.
- (b) 0.05 ml of a solution of the test chemical to give the appropriate dose.
- (c) 0.1 ml - 0.2 ml of indicator organism(s).
- (d) 0.50 ml of 0.2M phosphate buffer, pH 7.4.

This mixture is swirled gently and then poured onto minimal agar plates (see 3B, Media). After the top agar has set, the plates are incubated at 37°C for approximately 2 days. The number of his⁺ revertant colonies growing on the plates is counted and recorded.

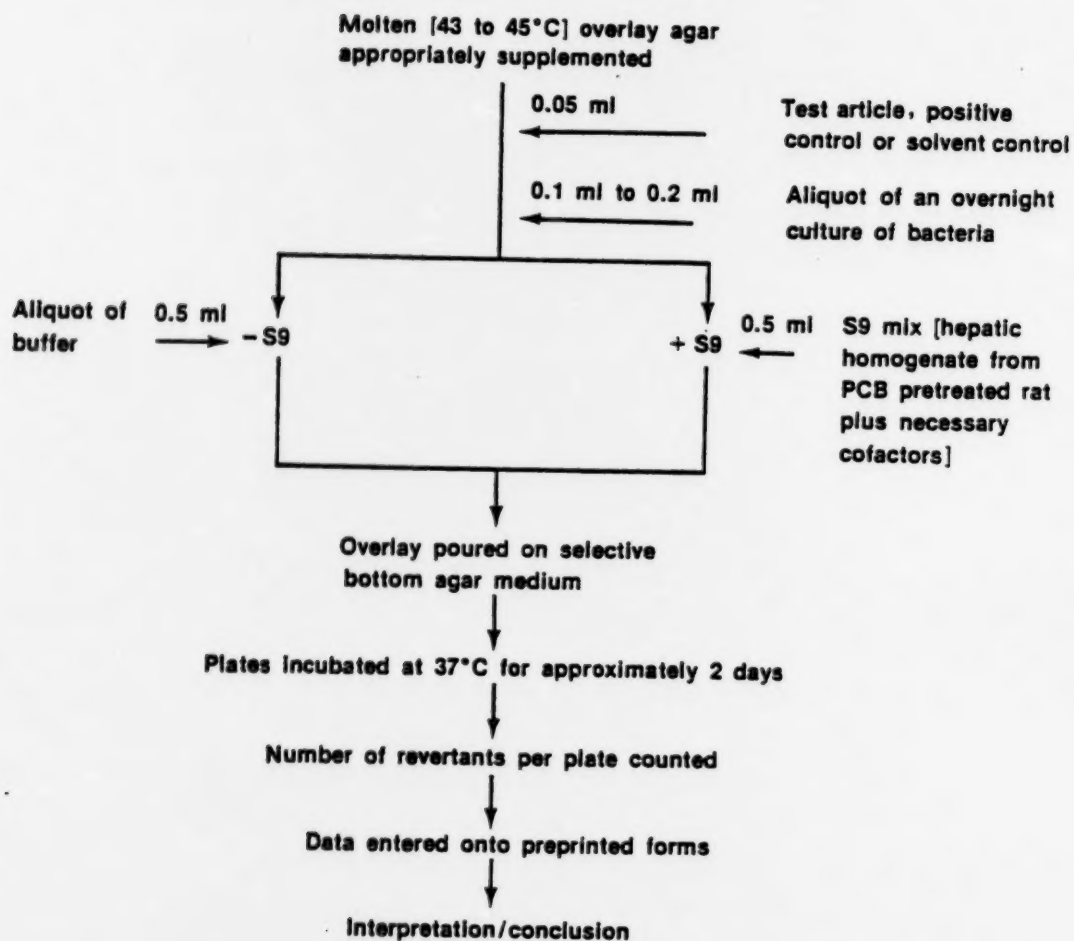
(2) Activation Assay

The activation assay is run concurrently with the nonactivation assay. The only difference is the addition of 0.5 ml of S9 mix (see 3C:2, Activation System) to the tubes in place of 0.5 ml of phosphate buffer which is added in nonactivation assays. All other details are similar to the procedure for nonactivation assays.

A detailed flow diagram for the plate incorporation assay is provided in Figure 1.

FIGURE 1

REVERSE MUTATION ASSAY
[Agar Incorporation Method]



4. EXPERIMENTAL DESIGN (Continued)

C. Control Compounds

A negative control consisting of the solvent used for the test material is performed in all cases. For negative controls, step 'b' of Nonactivation Assays is replaced by 0.05 ml of the solvent. The negative controls are employed for each indicator strain and are performed in the absence and presence of S9 mix. The solvent used to prepare the stock solution of the test material is given in the Results section of this report. All dilutions of the test material are made using this solvent. The amount of solvent used is equal to the maximum volume used to give the appropriate test dose.

Specific positive control compounds known to revert each strain are also used in the assays. The concentrations and specificities of these compounds to specific strains are given in the following table:

Assay	Chemical	Solvent	Concentration per Plate (μ g)	<u>Salmonella</u> Strains
Nonactivation	Sodium azide	Water	1	TA-1535, TA-100
	2-Nitrofluorene (NF)	Dimethyl- sulfoxide	10	TA-98
	9-aminoacridine (9AA)	Ethanol	50	TA-1537
Activation	2-anthramine (ANTH)	Dimethyl- sulfoxide	2.5	For all strains

5. EVALUATION CRITERIA

Statistical methods are not currently used and evaluation is based on the criteria included in this protocol.

Plate test data consist of direct revertant colony counts obtained from a set of selective agar plates seeded with populations of mutant cells suspended in a semisolid overlay. Because the test material and the cells are incubated in the overlay for approximately 2 days and a few cell divisions occur during the incubation period, the test is semiquantitative in nature. Although these features reduce the quantitation of result, they provide certain advantages not contained in a quantitative suspension test:

- The small number of cell divisions permits potential mutagens to act on replicating DNA, which is often more sensitive than nonreplicating DNA.
- The combined incubation of the test article and the cells in the overlay permits constant exposure of the indicator cells for approximately 2 days.

A. Surviving Populations

Plate test procedures do not permit exact quantitation of the number of cells surviving chemical treatment. At low concentrations of the test material, the surviving population on the treatment plates is essentially the same as that on the negative control plate. At high concentrations, the surviving population is usually reduced by some fraction. Our protocol will normally employ several doses ranging over two or three log concentrations. This does not apply to spot tests and tests performed on fabrics and like materials which are tested at a single concentration.

B. Dose-Response Phenomena

The demonstration of dose-related increases in mutant counts is an important criterion in establishing mutagenicity. A factor that might modify dose-response results for a mutagen would be the selection of doses that are too low (usually mutagenicity and toxicity are related). If the highest dose is far lower than a toxic concentration, no increases may be observed over the dose range selected. Conversely, if the lowest dose employed is highly cytotoxic, the test material may kill any mutants that are induced, and the test material will not appear to be mutagenic.

5. EVALUATION CRITERIA (Continued)

C. Control Tests

Positive and negative control assays are conducted with each experiment and consist of direct-acting mutagens for nonactivation assays and mutagens requiring metabolic biotransformation in activation assays. Negative controls consist of the test material solvent in the overlay agar together with the other essential components. The negative control plate for each strain gives a reference point to which the test data is compared. The positive control assay is conducted to demonstrate that the test systems are functional with known mutagens.

D. Evaluation Criteria for Ames Assay

Because the procedures used to evaluate the mutagenicity of the test material are semiquantitative, the criteria used to determine positive effects are inherently subjective and are based primarily on a historical data base. Most data sets are evaluated using the following criteria:

(1) Strains TA-1535, TA-1537

If the solvent control value is within the normal range, a test material producing a positive response equal to three times the solvent control value is considered mutagenic.

(2) Strains TA-98 and TA-100

If the solvent control value is within the normal range, a test material producing a positive response equal to twice the solvent control value for TA-98 and TA-100 is considered mutagenic.

(3) Pattern

Because TA-1535 and TA-100 are both derived from the same parental strain (G-46) and because TA-1538 and TA-98 are both derived from the same parental strain (D3052), to some extent there is a built-in redundancy in the microbial assay. In general, the two strains of a set respond to the same mutagen and such a pattern is sought. Generally, if a strain responds to a mutagen in nonactivation tests, it will do so in activation tests.

(4) Reproducibility

If a test material produces a response in a single test which cannot be reproduced in additional runs, the initial positive test data lose significance.

5. EVALUATION CRITERIA (Continued)

E. Evaluation Criteria for Toxicity

(1) Complete toxicity

When there are no revertants observed on the plate(s) treated with the test compound, the test compound is defined as toxic to all or any of the indicator strains at that (those) particular dose(s).

(2) Slight toxicity

When there are fifty or less percent revertants on the plate(s) treated with the test compound as compared to the solvent control plate(s), the test compound is defined as slightly toxic to all or any of the indicator strains at that (those) particular dose(s).

F. Relation Between Mutagenicity and Carcinogenicity

It must be emphasized that the Ames Salmonella/Microsome Plate Assay is not a definitive test for chemical carcinogens. It is recognized, however, that correlative and functional relations have been demonstrated between these two endpoints. The results of comparative tests on 300 chemicals by McCann et. al.¹ show an extremely good correlation between results of microbial mutagenesis tests and in vivo rodent carcinogenesis assays.

All evaluations and interpretation of the data to be presented in the final report will be based only on the demonstration, or lack, of mutagenic activity.

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Q.A. Inspection Statement
(reference 21 CFR 58.35(b)(7))

PROJECT 20988

LBI Assay No. 5397

TYPE of STUDY AMES PLATE TEST

This final study report was reviewed by the LBI Quality Assurance Unit on 12/3/80. A report of findings was submitted to the Study Director and to Management on 12/3/80.

The short-term nature of this study precluded inspection while it was in process. The Quality Assurance Unit inspects an in-process study of this type approximately once per month to assure that no significant problems exist that are likely to affect the integrity of this type of study.

Mitchell S. Elie
Auditor, Quality Assurance Unit

GENETICS ASSAY NO. 5394

LBI SAFETY NO. 6378

CYTOTOXIC EVALUATION OF
SAMPLE # 1452
(WATER TANK DRAIN, END OF TEST)

IN THE
RODENT CELL (CHO)
CLONAL TOXICITY ASSAY

FINAL REPORT

SUBMITTED TO:
ACUREX CORPORATION
485 CLYDE AVENUE
MOUNTAIN VIEW, CA 94042

SUBMITTED BY:
LITTON BIONETICS, INC.
5516 NICHOLSON LANE
KENSINGTON, MARYLAND 20795

LBI PROJECT NO. 20933
REPORT DATE: DECEMBER 1980

PREFACE

This report contains a summary of the data compiled during the evaluation of the test compound. The report is organized to present the results in a concise and easily interpretable manner. The first part contains items I - IX. Items I - IV provide sponsor and compound identification information, type of assay, and the assay design reference number. All assay design references indicate a standard procedure described in the Litton Bionetics, Inc. "Screening Program for the Identification of Potential Mutagens and Carcinogens." Item V provides the initiation and completion dates for the study, and Item VI provides identification of supervisory personnel. Item VII identifies the tables and figures containing the data used by the study director in interpreting the test results. The interpretation itself is in Item VIII. Item IX provides the conclusion and evaluation.

The second part of the report, entitled Assay Design, describes the materials and procedures employed in conducting the assay. This part of the report also contains any appendices, as well as evaluation criteria used by the study director. The evaluation criteria are included to acquaint the sponsor with the methods used to develop and analyze the test results.

All test and control results presented in this report are supported by fully documented raw data which are permanently maintained in the files of the Department of Genetics and Cell Biology or in the archives of Litton Bionetics, Inc., 5516 Nicholson Lane, Kensington, Maryland, 20795.

Copies of raw data will be supplied to the sponsor upon request.

- I. SPONSOR: Acurex Corporation
- II. MATERIAL (TEST COMPOUND): GENETICS ASSAY NUMBER: 5394
- A. Identification: Sample #1452 (Man Test 5/21/80, #5 Gal,
Water Tank Drain, End of Test)
- B. Date Received: October 6, 1980
- C. Physical Description: Clear, colorless liquid
- III. TYPE OF ASSAY: Rodent Cell (CHO) Clonal Toxicity Assay
- IV. ASSAY DESIGN NUMBER: 442
- V. STUDY DATES:
- A. Initiation: November 19, 1980
- B. Completion: December 1, 1980
- VI. SUPERVISORY PERSONNEL:
- A. Study Director: Brian C. Myhr, Ph.D.
- B. Laboratory Supervisor: Robert Young, M.S.
- VII. RESULTS:
- The data are presented in Table 1 on page 3 and in Figure 1 on page 4.
- VIII. INTERPRETATION OF RESULTS:
- The test material, Sample No. 1452, was supplied as a colorless, aqueous solution that did not contain any obvious suspended material. A portion of the sample (18 ml) was combined with 10X F10 medium and supplements to yield 30 ml of culture medium containing the test material at a concentration of 600 μ l/ml. This was the highest concentration applied to the CHO cells. Lower concentrations were achieved by serial dilution with F10 culture medium. The treatments were initiated by replacing the F12 medium in the cell cultures with F10 medium containing the test material. After the 24 hour exposure period, the cultures were returned to F12 medium and incubated for colony development. The test material did not cause any visible precipitation of culture medium components, but a shift to a slightly acidic pH of 6.26 was observed for the 600 μ l/ml concentration. The pH remained normal (above 7.0) for the other test concentrations.

VIII. INTERPRETATION OF RESULTS: (continued)

Two trials of the assay were performed. The first trial was considered insufficient for evaluation because the average cloning efficiency of the negative controls was 67.4%, which was slightly below the 70% criterion used for an acceptable assay. The results of the second trial are presented in Table 1 and Figure 1.

Seven test concentrations from 6 $\mu\text{l/ml}$ to 600 $\mu\text{l/ml}$ were evaluated for their effects on colony survival. As shown in Table 1, complete lethality was obtained for treatments in the 60 to 600 $\mu\text{l/ml}$ concentration range. In contrast, the treatments with 6 to 30 $\mu\text{l/ml}$ were essentially nontoxic to the cells. Thus, an extremely sharp survival curve was obtained, as shown in Figure 1, with an EC50 apparently located at 45 $\mu\text{l/ml}$. [In the first trial (results not shown), an EC50 value of 52 $\mu\text{l/ml}$ was obtained for a somewhat broader survival curve]. The EC50 was therefore located at the upper end of the 6-60 $\mu\text{l/ml}$ concentration range, which is the range defined for moderate toxicity for aqueous liquids (see Evaluation Criteria). Since complete lethality was obtained at the boundary between moderate and low toxicity, an evaluation of moderate toxicity to CHO cells appeared appropriate.

IX. CONCLUSIONS:

The test material, Sample No. 1452, was evaluated as having moderate toxicity in the CHO Clonal Toxicity Assay. The EC50 value was 45 $\mu\text{l/ml}$.

Submitted by:

Study Director:

Brian C. Myhr
Brian C. Myhr, Ph.D.
Section Chief
Mammalian Genetics
Department of Genetics
and Cell Biology

12/3/80
Date

Reviewed by:

David J. Brusick
David J. Brusick, Ph.D.
Director
Department of Genetics
and Cell Biology

12/3/80
Date

TABLE 1

RODENT CELL (CHO) CLONAL TOXICITY ASSAY

Sample Identity: Sample #1452. (Water EC50 Value: 45 μ l/ml
Tank Drain, End of Test) Toxicity
 Description of Sample: Clear, colorless Classification: Moderate
aqueous liquid pH Alterations: pH 6.26 at 600 μ l/ml
 LBI Assay No.: 5394 Comments on Treatment: Normal pH
 Date Received: October 6, 1980 for treatments with 6 to 300 μ l/ml
 Test Date: November 25, 1980 (Trial 2)
 Vehicle: None
 Cell Type: CHO-K1
 Cells Seeded per Dish: 200

CLONAL TOXICITY DATA

Sample	Applied Concentration μ l/ml	Dish #1	Dish #2	Dish #3	Average Count	Relative Survival %	Cloning Efficiency %
NC	---	187	193	187	189.0	100.0	94.5
Test	6.0	190	219	183	197.3	104.4	
Test	10.0	214	210	181	201.7	106.7	
Test	30.0	167	190	178	178.3	94.3	
Test	60.0	0	0	0	0	0	
Test	100.0	0	0	0	0	0	
Test	300.0	0	0	0	0	0	
Test	600.0	0	0	0	0	0	

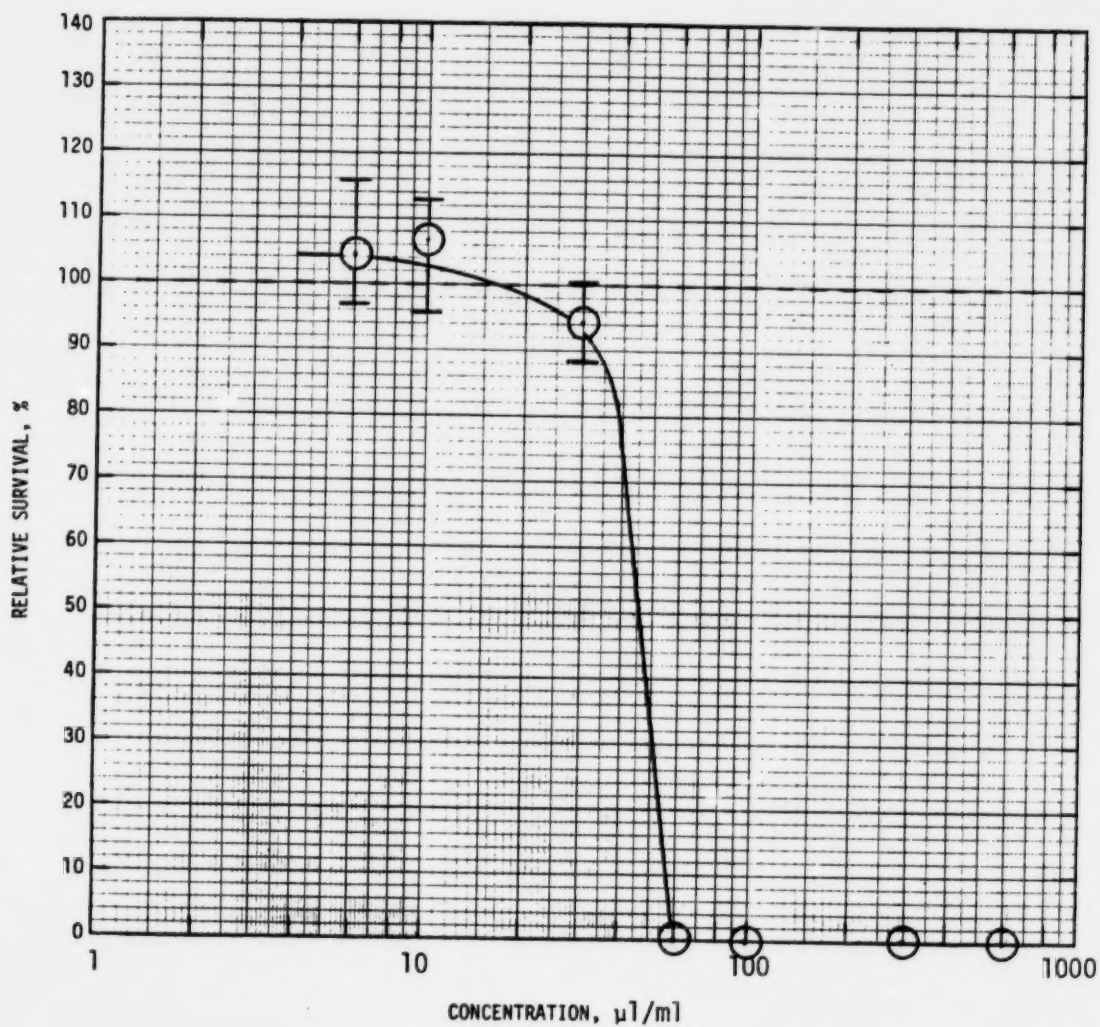
NC = Negative Control, F12 medium.

FIGURE 1

RODENT CELL (CHO) CLONAL TOXICITY ASSAY

EC₅₀ DETERMINATION

SAMPLE #1452 (WATER TANK DRAIN, END OF TEST)



ASSAY DESIGN NO. 442

RODENT CELL CLONAL (CHO) TOXICITY ASSAY

This assay conforms to one of the bioassay tests in the US Environmental Protection Agency (EPA) IERL - RTP Procedures Manual: Level 1 Environmental Assessment Biological Tests (September, 1980). Level 1 bioassays obtain preliminary information on the harmful biological effects of chemicals found in industrial feed and waste streams by using mammalian cells in culture to measure metabolic impairment and cellular death.

1. OBJECTIVE

The objective of this assay is to determine the concentration of a test article that will reduce the colony forming ability of Chinese hamster cells by 50% after a 24-hour exposure. This concentration is referred to as the EC_{50} value.

2. MATERIALS

A. Indicator Cells

The indicator cells used in this study are Chinese hamster CHO-K1 cells (American Type Culture Collection No. CCL 61). This cell type was derived from ovarian tissue and has spontaneously transformed to a stable, hypodiploid line of rounded, fibroblastic cells with unlimited growth potential. Monolayer cultures have a fast doubling time of 11 to 14 hours, and untreated cells can normally be cloned with an efficiency of 80% or greater. Permanent stocks are maintained in liquid nitrogen and laboratory cultures are maintained by serial subculturing. Laboratory cultures are periodically checked by culturing methods for the absence of mycoplasma contamination. This test system is specified by the IERL- RTP Procedures Manual.

B. Medium

The CHO-K1 cell line has an absolute requirement for proline and therefore must be maintained in a culture medium containing sufficient amounts of this amino acid. Ham's F12 medium, which contains $3 \times 10^{-4}M$ L-proline, is used, supplemented with 10% fetal bovine serum, 100 units per milliliter of penicillin, 100 μg streptomycin per milliliter, and 0.5 μg amphotericin B (Fungizone) per milliliter.

C. Controls

Untreated cells are cloned to establish the control cloning efficiency. If the test article is dissolved in an organic solvent (usually dimethylsulfoxide), cells exposed to solvent in the medium

2. MATERIALS (Continued)

are cloned to provide the reference cloning efficiency for the effect of the test article. The final concentration of solvent in the growth medium is generally 1% or less. All controls are performed in triplicate.

D. Sample Forms

Solid samples are tested as a solution or are ground to fine particles (less than 5 μm) and tested as a suspension in growth medium. Dry particulate articles, aqueous liquids, suspensions and slurries are added directly to the growth medium and tested as a suspension or solution. Liquids containing less than 0.2% organic solvent are generally tested directly; samples dissolved in organic solvents are solvent exchanged in dimethylsulfoxide (DMSO) before testing. Original sample volumes may be reduced a maximum of 10-fold during solvent exchange, and the concentration factor is used to convert assayed volumes into equivalent original sample volumes. All sample manipulations are performed as described in the IERL-RTP Level 1 Procedures Manual.

3. EXPERIMENTAL DESIGN

A. Dose Selection

Unless the approximate toxicity is already known or the sample size is limiting, the following dose ranges are tested for different sample forms. Dry particulate articles are dissolved or suspended in growth medium and tested at five dose levels from 1000 $\mu\text{g/ml}$ to 10 $\mu\text{g/ml}$. Aqueous samples, suspensions or slurries are tested from 600 $\mu\text{liters/ml}$ to 6 $\mu\text{liters/ml}$ in five dose steps. Samples that are solvent exchanged into DMSO are tested from 20 $\mu\text{liters/ml}$ to 0.2 $\mu\text{liters/ml}$, also in five dose steps. Solvent concentrations are 1% for all dose levels except the maximum applicable dose (MAD) of 20 $\mu\text{l/ml}$, which contains 2% solvent. A second dose study is performed with an appropriate dose range if the EC_{50} has not been properly located in the initial test; EC_{50} values greater than 1,000 $\mu\text{g/ml}$, 600 μliters of aqueous sample/ml or 20 μliters nonaqueous sample/ml are not determined.

B. Clonal Toxicity Assay

Cells from monolayer stock cultures in logarithmic growth phase are trypsinized, counted by hemacytometer and reseeded into a series of 60- or 100-mm culture dishes at 200 cells per dish. The cultures are incubated for 6 to 16 hours at 37°C to allow attachment of the cells and recovery of growth rate.

Test article is then applied (three dishes per dose), and the cultures returned to the incubator. If the test article causes a color change, an additional dish is treated with the high dose and the pH of the

3. EXPERIMENTAL DESIGN (Continued)

medium is determined. The pH is also recorded for the highest dose which results in a slight color change. After a 24-hour exposure period the medium is aspirated and the cells washed with Dulbecco's phosphate buffered saline (PBS; prewarmed to 37°C). The pH of the discarded medium for which initial pH measurements were made is again recorded. Fresh medium is placed on each culture and incubation continued for an additional 6 days to allow colony development. Medium is drained from the cultures and the surviving colonies are washed with PBS, fixed in methanol, and stained with Giemsa. Colony counting is performed by eye; colonies smaller than 50 to 100 cells are not counted.

4. ASSAY ACCEPTANCE CRITERIA

The assay is considered acceptable for evaluation of the test results if the following criteria are met:

- The average cloning efficiency of the CHO-K1 cells in the negative controls is 70% or greater, but not exceeding 115%.
- The distribution of colonies in the treated cultures is generally uniform over the surface of the culture dish.
- The data points for each test concentration critical to the location of the EC_{50} are the averages of at least two treated cultures.
- A sufficient number of test concentrations are available to clearly locate the EC_{50} within a toxicity region as defined under Assay Evaluation Criteria.
- If the EC_{50} value is greater than 1000 µg/ml, 600 µliters of aqueous sample/ml, or 20 µliters of nonaqueous sample/ml, the plotted curve does not exceed 110% of the negative control.

5. ASSAY EVALUATION CRITERIA

The screened doses, pH values (if appropriate), colony counts, percent survivals (colony counts relative to control colony counts) and EC_{50} values are provided. The percent survival is plotted as a function of applied concentration and the EC_{50} value determined graphically by fitting a curve by eye through the data points. The EC_{50} is used to rank the test material using the standard evaluation criteria defined in the table below.

Sorbent extracts of known organic content are evaluated as nonaqueous liquids and in terms of their solid contents (µg organics/ml), and are ranked using the more sensitive parameter. Where data on the original liquid or gas sample volumes is provided by the Sponsor, the EC_{50} value is calculated in terms of these equivalent volumes per milliliter of culture medium; however, no evaluations are made as yet on this basis.

5. ASSAY EVALUATION CRITERIA (Continued)

Toxicity ^a	Solids (EC ₅₀ in µg/ml)	Aqueous Liquids (EC ₅₀ in µl/ml)	Nonaqueous Liquids ^b (EC ₅₀ in µl/ml)
High	<10	<6	<.2
Moderate	10 to 100	6 to 60	.2-2
Low	100 to 1000	60 to 600	2-20
Not Detectable	>1000	>600	>20

^aEvaluation criteria formulated by Litton Bionetics, Inc. for IERL-RTP Procedures Manual: Level 1 Environmental Assessment Biological Tests.

^bCriteria for nonaqueous liquids are tentative and under evaluation.

6. RECORDS TO BE MAINTAINED

All raw data, protocols, protocol modifications, test article weight and dispensation records and correspondence between LBI and the Sponsor are being maintained in a central file within the Department of Genetics and Cell Biology. These records will be filed under departmental assay number and held up to 2 years following submission of the final report to the Sponsor. After 2 years they will be transferred to the LBI archives for permanent storage.

Q.A. Inspection Statement
(reference 21 CFR 58.35(b)(7))

PROJECT 20993

LBI Assay No. 5394

TYPE of STUDY Rodent Cell (CHO) Clonal Toxicity Assay

This final study report was reviewed by the LBI Quality Assurance Unit on December 3, 1980. A report of findings was submitted to the Study Director and to Management on December 3, 1980.

The short-term nature of this study precluded inspection while it was in process. The Quality Assurance Unit inspects an in-process study of this type approximately once per month to assure that no significant problems exist that are likely to affect the integrity of this type of study.

Patrice M. Cuccaro

Auditor, Quality Assurance Unit

GENETICS ASSAY NO. 5394

LBI SAFETY NO. 6378

MUTAGENICITY EVALUATION OF
SAMPLE #1452

IN THE
AMES SALMONELLA/MICROSOME
PLATE TEST

FINAL REPORT

SUBMITTED TO:

ACUREX CORPORATION
485 CLYDE AVENUE
MOUNTAIN VIEW, CA 94042

SUBMITTED BY:

LITTON BIONETICS, INC.
5516 NICHOLSON LANE
KENSINGTON, MARYLAND 20795

LBI PROJECT NO. 20988

REPORT DATE: DECEMBER, 1980

PREFACE

This report contains a summary of the data compiled during the evaluation of the test compound. The report is organized to present the results in a concise and easily interpretable manner. The first part contains items I-IX. Items I-IV provide sponsor and compound identification information, type of assay, and the protocol reference number. All protocol references indicate a standard procedure described in the Litton Bionetics, Inc. "Screening Program for the Identification of Potential Mutagens and Carcinogens." Item V provides the initiation and completion dates for the study, and Item VI provides identification of supervisory personnel. Item VII identifies the tables and/or figures containing the data used by the study director in interpreting the test results. The interpretation itself is in Item VIII. Item IX provides the conclusion and evaluation.

The second part of the report describes the materials and procedures employed in conducting the assay. This part of the report also contains evaluation criteria used by the study director, and any appendices. The evaluation criteria are included to acquaint the sponsor with the methods used to develop and analyze the test results.

All test and control results presented in this report are supported by fully documented raw data which are permanently maintained in the files of the Department of Genetics and Cell Biology or in the archives of Litton Bionetics, Inc., 5516 Nicholson Lane, Kensington, Maryland 20795.

- I. SPONSOR : Acurex Corporation
- II. MATERIAL (TEST COMPOUND): GENETIC ASSAY NUMBER: 5394
- A. Identification: Sample Number 1452 and its Extract
- B. Date Received : October 6, 1980
- C. Physical Description: Neat Sample: Colorless Liquid
Extract : Green Liquid
- III. TYPE OF ASSAY : Ames Salmonella/microsome Mutagenesis Assay
- IV. PROTOCOL NUMBER: 401 (EPA-LEVEL I)
- V. STUDY DATES:
- A. Initiation: November 21, 1980
- B. Completion: November 25, 1980
- VI. SUPERVISORY PERSONNEL:
- A. Study Director: D.R. Jagannath, Ph.D.
- VII. RESULTS:
- The results of this are presented in Tables 1 and 2.
- VIII. INTERPRETATION OF RESULTS:
- The test material, Sample No. 1452 was received as a clear colorless liquid (1 gallon) with no visible suspended particulates. An aliquot of the neat sample (3250 ml) was concentrated 500 fold by passing through the XAD-2 resin column at a flow rate of 3 ml per minute. The resin was then extracted with methylene chloride in a Soxhlet apparatus for 24 h. The extract (250 ml) was concentrated to approximately 5 ml using a Kuderna Concentrator. The concentrated extract was then solvent exchanged by sequential addition of Dimethylsulfoxide (DMSO) and evaporation of residual methylene chloride in a warm water bath under a stream of nitrogen. Final sample size in DMSO was 6.5 ml which represents a 500 fold concentration factor. The concentrate was a clear solution with a pale blue/green color.
- The test materials, neat sample #1452 and its extract were examined for mutagenic activity in the Ames Salmonella assay in the presence and absence of liver microsomal enzymes from Aroclor induced rats.
- Solvent controls, distilled water for the neat sample and DMSO for the extract of the sample, and specific positive controls were also assayed concurrently with the test materials.

DOSE RANGE: The dose range employed for the neat sample was from 10.0 ul to 200.0 ul per plate and for the extract it was from 5.0 ul to 100.0 ul per plate (due to the limited amount of the extract). The organic content of the sample was not supplied by the sponsor.

TOXICITY: The test materials, neat sample and its extract were not toxic to any of the strains used in this assays.

RESULTS:

The results of the tests conducted on the test material, neat sample, in the absence of an activation system was negative. However, the extract of the sample exhibited genetic activity with the strain TA-98 in the absence of an activation system. The response was slightly above two times the background level and was observed at the highest concentration of 100 ul per plate.

The results of the tests conducted on the test materials neat sample and its extract were all negative in the presence of rat liver activation system.

Dose-related increases in the number of revertants were also observed with the base-pair substitution strain TA-100 in the non-activation and activation assays performed on the extract. However these increases did not meet the criteria for a positive response.

IX. CONCLUSION:

The test material, neat sample #1452 and its extract were tested in the Ames/Salmonella assay and were compared for their mutagenic activities. The neat sample did not exhibit genetic activity in any of the assays conducted in this evaluation and was considered not mutagenic under these conditions. However, the extract of the sample #1452 exhibited genetic activity with the strain TA-98 in the nonactivation assays conducted in this evaluation and is considered as mutagenic. Based on the EPA's "Definition of Toxicity Categories for Health Effects Tests" the mutagenic activity of the neat sample #1452 is classified as 'ND' and the mutagenic activity of the extract of the sample #1452 is classified as 'low'. Since organic content of the sample was not available the specific activity has not been calculated.

Submitted by:

Reviewed by:

Study Director

for, Brusick C. Myler 12/3/80
David J. Brusick, Ph.D. Date
Director
Department of Genetics
and Cell Biology

D.R. Jagannathan 12/3/80
D.R. Jagannathan, Ph.D. Date
Section Chief
Submammalian Genetics
Department of Genetics
and Cell Biology

RESULTS

TABLE 1

A. NAME OR CODE DESIGNATION OF THE TEST COMPOUND: SAMPLE #1452 (EXTRACT)
 B. SOLVENT: DM2C
 C. TEST INITIATION DATE: 11/21/80
 D. TEST COMPLETION DATE: 11/25/80
 E. S-Q LOT#: DM009
 NOTE: CONCENTRATIONS ARE GIVEN IN MICROLITERS PER PLATE

TEST	SPECIES	TISSUE	REPLICANTS PER PLATE											
			TA-1535			TA-1537			TA-98			TA-100		
			1	2	3	1	2	3	1	2	3	1	2	3
NONACTIVATION														
SOLVENT CONTROL	---	---	28	39		13	11		25	25		126	176	
POSITIVE CONTROL**	---	---	620	662		797	848		1013	890		701	654	
TEST COMPOUND														
10-000000 UL	---	---	31	28		8	11		20	22		142	139	
25-000000 UL	---	---	31	27		9	9		38	20		146	142	
50-000000 UL	---	---	32	31		16	9		22	22		115	132	
100-000000 UL	---	---	29	20		8	8		29	33		128	150	
200-000000 UL	---	---	22	19		0	12		33	28		139	128	
ACTIVATION														
SOLVENT CONTROL	RAT	LIVER	27	31		10	16		46	58		145	135	
POSITIVE CONTROL**	RAT	LIVER	163	164		262	210		1302	1263		1585	1523	
TEST COMPOUND														
10-000000 UL	RAT	LIVER	27	31		10	7		36	28		100	78	
25-000000 UL	RAT	LIVER	27	19		4	3		29	33		77	85	
50-000000 UL	RAT	LIVER	42	15		5	10		26	17		87	87	
100-000000 UL	RAT	LIVER	28	16		8	9		26	26		82	76	
200-000000 UL	RAT	LIVER	21	14		7	4		16	24		106	93	

** TA-1535 SODIUM AZIDE
 TA-1537 9-ANTHRACIDINE
 TA-98 2-NITROFLUORENE
 TA-100 SODIUM AZIDE
 SOLVENT 200 UL/PLATE

... TA-1535 2-ANTHRACINE 2.5 UG/PLATE
 TA-1537 2-ANTHRACINE 2.5 UG/PLATE
 TA-98 2-ANTHRACINE 2.5 UG/PLATE
 TA-100 2-ANTHRACINE 2.5 UG/PLATE

RESULTS

TABLE 2

A. NAME OF CODE DESIGNATION OF THE TEST COMPOUND: SAMPLE 814-2 (EXTRACT)
 B. SOLVENT: DMSO
 C. TEST INITIATION DATES: 11/21/80
 D. TEST COMPLETION DATE: 11/25/80
 E. S-Y LOT#: 08009
 NOTE: CONCENTRATIONS ARE GIVEN IN MICROLITERS PER PLATE

TEST	SPECIES	TISSUE	REVERTANTS PER PLATE											
			TA-1535			TA-1537			TA-98			TA-100		
			1	2	3	1	2	3	1	2	3	1	2	3
NONACTIVATION														
SOLVENT CONTROL	---	---	32	35	7	7	7	51	25	144	141			
POSITIVE CONTROL**	---	---	620	662	797	800	1013	800		701	654			
TEST COMPOUND														
5.000000 UL	---	---	11	14	10	4		30	33	164	162			
10.000000 UL	---	---	17	22	11	13		28	31	174	173			
25.000000 UL	---	---	20	22	17	15		38	32	178	164			
50.000000 UL	---	---	23	23	16	14		64	46	213	199			
100.000000 UL	---	---	27	14	14	20		69	46	233	226			
ACTIVATION														
SOLVENT CONTROL	RAT	LIVER	34	27	18	9		39	37	153	143			
POSITIVE CONTROL***	RAT	LIVER	163	164	262	210		1302	1263	1565	1523			
TEST COMPOUND														
5.000000 UL	RAT	LIVER	42	42	7	8		33	27	142	114			
10.000000 UL	RAT	LIVER	53	40	7	14		41	43	121	154			
25.000000 UL	RAT	LIVER	26	34	13	19		39	27	172	142			
50.000000 UL	RAT	LIVER	24	44	14	23		44	49	204	193			
100.000000 UL	RAT	LIVER	32	34	20	24		57	53	239	182			

**

TA-1535 SODIUM AZIDE
 TA-1537 9-AMINOCARBOINE
 TA-98 2-NITROFLUORENE
 TA-100 SODIUM AZIDE
 SOLVENT 100 UL/PLATE

10 UC/PLATE
 10 UC/PLATE
 10 UC/PLATE
 10 UC/PLATE

TA-1535 2-ANTHRACINE
 TA-1537 2-ANTHRACINE
 TA-98 2-ANTHRACINE
 TA-100 2-ANTHRACINE
 2.5 UC/PLATE
 2.5 UC/PLATE
 2.5 UC/PLATE
 2.5 UC/PLATE

TABLE A

DEFINITION OF TOXICITY CATEGORIES FOR HEALTH EFFECTS ASSAYS*

Assay ^a	Activity Measured ^b	Sample Type	MAD ^d	Units	Range of Concentration or Dosage		
					High	Moderate	Low
Ames	MEC (mutagenesis)	S AL, NAL	5 200	mg/plate μl/plate	<0.05	0.05-0.5 2-20	0.5-5 20-200
RAM	EC ₅₀ (lethality)	S	1	mg/ml	<0.01	0.01-0.1	0.1-1
		AL NAL	600 20	μl/ml μl/ml	<6 <0.2	6-60 0.2-2	60-600 2-20
CHO	EC ₅₀ (lethality)	S	1	mg/ml	<0.01	0.01-0.1	0.1-1
		AL NAL	600 20	μl/ml μl/ml	<6 <0.2	6-60 0.2-2	60-600 2-20
MAT	LD ₅₀ (lethality and toxic signs)	S	5	gm/kg	<0.05	0.05-0.5	0.5-5
		AL, NAL	5	ml/kg	<0.05	0.5-0.5	0.5-5

^aStandard test abbreviations are as follows:

Ames: Ames Salmonella/microsome mutagenesis assay
RAM: Rabbit alveolar macrophage cytotoxicity assay
CHO: Rodent cell clonal toxicity assay
MAT: Acute in vivo test in rodents (whole animal test)

^bStandard abbreviations for measured endpoints are as follows:

MEC: Minimum effective concentration
EC₅₀: Calculated concentration expected to produce effect in 50 percent of population
LD₅₀: Calculated dose expected to kill 50 percent of population

C_S = Solid, AL = Aqueous liquid, NAL = Nonaqueous liquid

^dMAD = Maximum applicable dose

*Evaluation criteria formulated by Litton Bionetics, Inc. for IERL-RTP Procedures Manual: Level 1 Environmental Assessment Biological Tests.

AMES SALMONELLA/MICROSOME PLATE ASSAY

1. OBJECTIVE

The objective of this study was to evaluate a test material for mutagenic activity in a bacterial assay with and without a mammalian S9 activation system.

2. RATIONALE

The Salmonella typhimurium strains used at LBI are all histidine auxotrophs by virtue of mutations in the histidine operon. When these histidine-dependent cells are grown in a minimal media petri plate containing a trace of histidine, only those cells that revert to histidine independence (his+) are able to form colonies. The trace amount of histidine allows all the plated bacteria to undergo a few divisions; this growth is essential for mutagenesis to occur. The his+ revertants are easily scored as colonies against the slight background growth. The spontaneous mutation frequency of each strain is relatively constant, but when a mutagen is added to the agar the mutation frequency is increased 2- to 100-fold. Cells which grow to form colonies on the minimal media petri plates are therefore assumed to have reverted, either spontaneously or by the action of a test substance to his+ genotype.

3. MATERIALS

A. Indicator Microorganisms

The Salmonella typhimurium strains used in this assay were obtained from Dr. Bruce Ames, University of California at Berkeley.¹⁻⁵ The following 4 strains were used:

Strain Designation	Gene Affected	Additional Mutations			Mutation Type Detected
		Repair	LPS	R Factor	
TA-1535	<u>his</u> G	Δ <u>uvr</u> B	<u>rfa</u>	-	Base-pair substitution
TA-1537	<u>his</u> C	Δ <u>uvr</u> B	<u>rfa</u>	-	Frameshift
TA-98	<u>his</u> D	Δ <u>uvr</u> B	<u>rfa</u>	pKM101	Frameshift
TA-100	<u>his</u> G	Δ <u>uvr</u> B	<u>rfa</u>	pKM101	Base pair substitution

3. MATERIALS (Continued)

The aforementioned strains have, in addition to the mutation in the histidine operon, a mutation (rfa-) that leads to defective lipopolysaccharide coat, a deletion that covers genes involved in the synthesis of vitamin biotin (bio-) and in the repair of ultraviolet (uv) - induced DNA damage (uvrB-). The rfa- mutation makes the strains more permeable to many large molecules. The uvrB- mutation decreases repair of some types of chemically or physically damaged DNA and thereby enhances the strain's sensitivity to some mutagenic agents. The resistant transfer factor plasmid (R factor) pKM101 in TA-98 and TA-100 is believed to cause an increase in error-prone DNA repair that leads to many more mutations for a given dose of most mutagens⁵. In addition, plasmid pKM101 confers resistance to the antibiotic ampicillin, which is a convenient marker to detect the presence of plasmid in the cells.

All indicator strains are kept at 4°C on minimal medium plates supplemented with a trace of biotin and an excess of histidine. The plates with plasmid-carrying strains contain in addition ampicillin (25 µg/ml) to ensure stable maintenance of plasmid pKM101. New stock culture plates are made as often as necessary from frozen master cultures or from single colony reisolates that were checked for their genotypic characteristics (his, rfa, uvrB, bio) and for the presence of plasmid. For each experiment, an inoculum from the stock culture plates is grown overnight at 37°C in nutrient broth (Oxoid CM67).

B. Media

The bacterial strains were cultured in Oxoid Media #2 (nutrient Broth). The selective medium was Vogel Bonner Medium E with 2% glucose⁷. The overlay agar consisted of 0.6% purified agar with 0.5 mM histidine, 0.05 mM biotin and 0.1 M NaCl according to the methods of Ames *et. al.*⁶

C. Activation System

(1) S9 Homogenate

A 9,000 x g supernatant prepared from Sprague-Dawley adult male rat liver induced by Aroclor 1254 (described by Ames *et. al.*⁶) was purchased from Bionetics Laboratory Products, Litton Bionetics, Inc. and used in this assay.

(2) S9 Mix

Components	Concentration per Milliliter S9 Mix
NADP (sodium salt)	4 µmoles
D-glucose-6-phosphate	5 µmoles
MgCl ₂	8 µmoles
KCl	33 µmoles
Sodium phosphate buffer pH 7.4	100 µmoles
Organ homogenate from rat liver (S9 fraction)	100 µliters

4. EXPERIMENTAL DESIGN

A. Dosage Selection

All tests are run at a minimum of four concentrations. In the Standard EPA Level I Ames assays, five dose levels of the test material, dissolved in a suitable solvent, are added to the test system. The standard test doses for the extracted material are 10, 25, 50, 100 and 200 μ liters per plate. The solids are tested up to 5 mg per plate and at lower concentrations of 2.5, 1, 0.5, 0.1 and 0.05 mg per plate. The samples are retested over a narrower range of concentrations with strains showing positive results if there is enough sample.

B. Mutagenicity Testing

The procedure used is based on the paper published by Ames et. al.⁶ and is performed as follows:

(1) Nonactivation Assay

To a sterile 13 x 100 mm test tube placed in a 43°C water bath the following is added in order:

- (a) 2.00 ml of 0.6% agar containing 0.05 mM histidine and 0.05 mM biotin.
- (b) 0.05 ml of a solution of the test chemical to give the appropriate dose.
- (c) 0.1 ml - 0.2 ml of indicator organism(s).
- (d) 0.50 ml of 0.2M phosphate buffer, pH 7.4.

This mixture is swirled gently and then poured onto minimal agar plates (see 3B, Media). After the top agar has set, the plates are incubated at 37°C for approximately 2 days. The number of his⁺ revertant colonies growing on the plates is counted and recorded.

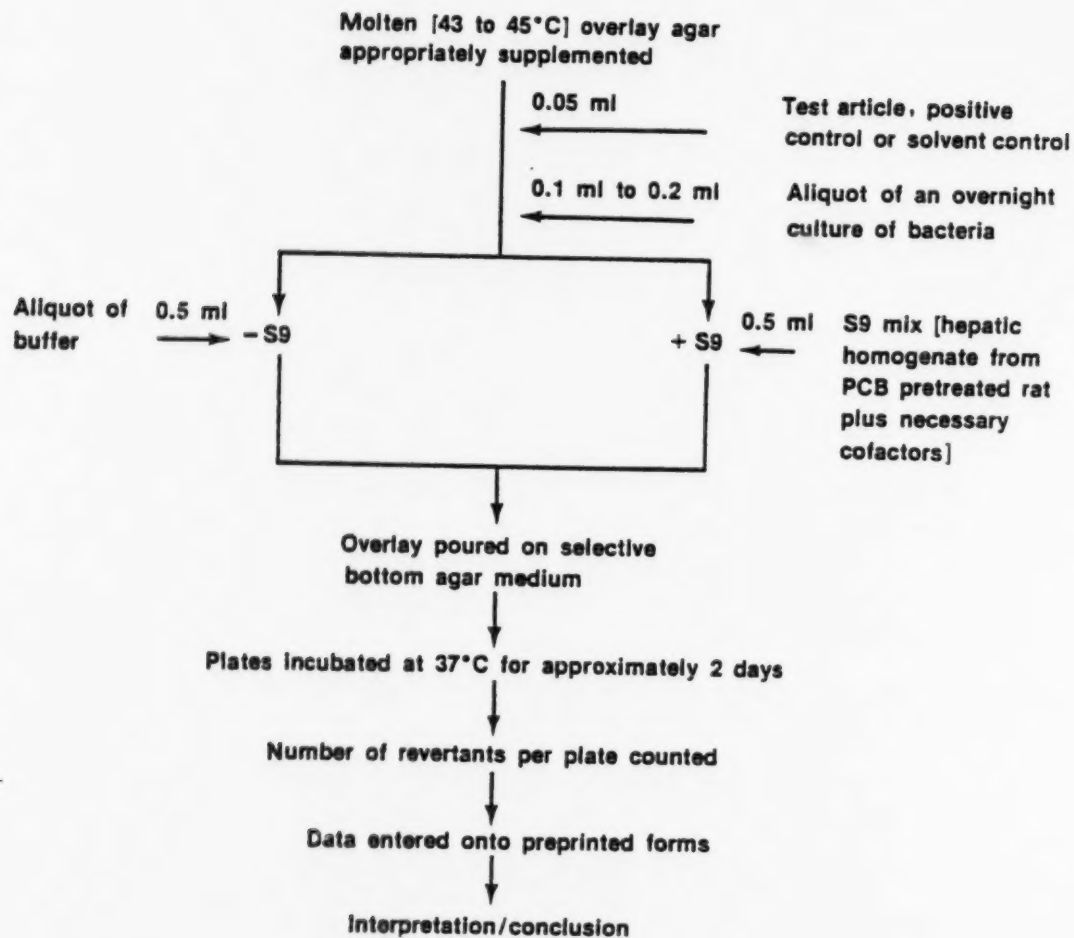
(2) Activation Assay

The activation assay is run concurrently with the nonactivation assay. The only difference is the addition of 0.5 ml of S9 mix (see 3C:2, Activation System) to the tubes in place of 0.5 ml of phosphate buffer which is added in nonactivation assays. All other details are similar to the procedure for nonactivation assays.

A detailed flow diagram for the plate incorporation assay is provided in Figure 1.

FIGURE 1

REVERSE MUTATION ASSAY
[Agar Incorporation Method]



4. EXPERIMENTAL DESIGN (Continued)

C. Control Compounds

A negative control consisting of the solvent used for the test material is performed in all cases. For negative controls, step 'b' of Nonactivation Assays is replaced by 0.05 ml of the solvent. The negative controls are employed for each indicator strain and are performed in the absence and presence of S9 mix. The solvent used to prepare the stock solution of the test material is given in the Results section of this report. All dilutions of the test material are made using this solvent. The amount of solvent used is equal to the maximum volume used to give the appropriate test dose.

Specific positive control compounds known to revert each strain are also used in the assays. The concentrations and specificities of these compounds to specific strains are given in the following table:

Assay	Chemical	Solvent	Concentration per Plate (μ g)	<u>Salmonella</u> Strains
Nonactivation	Sodium azide	Water	1	TA-1535, TA-100 TA-98
	2-Nitrofluorene (NF)	Dimethyl- sulfoxide	10	
	9-aminoacridine (9AA)	Ethanol	50	TA-1537
Activation	2-anthramine (ANTH)	Dimethyl- sulfoxide	2.5	For all strains

5. EVALUATION CRITERIA

Statistical methods are not currently used and evaluation is based on the criteria included in this protocol.

Plate test data consist of direct revertant colony counts obtained from a set of selective agar plates seeded with populations of mutant cells suspended in a semisolid overlay. Because the test material and the cells are incubated in the overlay for approximately 2 days and a few cell divisions occur during the incubation period, the test is semiquantitative in nature. Although these features reduce the quantitation of result, they provide certain advantages not contained in a quantitative suspension test:

- The small number of cell divisions permits potential mutagens to act on replicating DNA, which is often more sensitive than nonreplicating DNA.
- The combined incubation of the test article and the cells in the overlay permits constant exposure of the indicator cells for approximately 2 days.

A. Surviving Populations

Plate test procedures do not permit exact quantitation of the number of cells surviving chemical treatment. At low concentrations of the test material, the surviving population on the treatment plates is essentially the same as that on the negative control plate. At high concentrations, the surviving population is usually reduced by some fraction. Our protocol will normally employ several doses ranging over two or three log concentrations. This does not apply to spot tests and tests performed on fabrics and like materials which are tested at a single concentration.

B. Dose-Response Phenomena

The demonstration of dose-related increases in mutant counts is an important criterion in establishing mutagenicity. A factor that might modify dose-response results for a mutagen would be the selection of doses that are too low (usually mutagenicity and toxicity are related). If the highest dose is far lower than a toxic concentration, no increases may be observed over the dose range selected. Conversely, if the lowest dose employed is highly cytotoxic, the test material may kill any mutants that are induced, and the test material will not appear to be mutagenic.

5. EVALUATION CRITERIA (Continued)

C. Control Tests

Positive and negative control assays are conducted with each experiment and consist of direct-acting mutagens for nonactivation assays and mutagens requiring metabolic biotransformation in activation assays. Negative controls consist of the test material solvent in the overlay agar together with the other essential components. The negative control plate for each strain gives a reference point to which the test data is compared. The positive control assay is conducted to demonstrate that the test systems are functional with known mutagens.

D. Evaluation Criteria for Ames Assay

Because the procedures used to evaluate the mutagenicity of the test material are semiquantitative, the criteria used to determine positive effects are inherently subjective and are based primarily on a historical data base. Most data sets are evaluated using the following criteria:

(1) Strains TA-1535, TA-1537

If the solvent control value is within the normal range, a test material producing a positive response equal to three times the solvent control value is considered mutagenic.

(2) Strains TA-98 and TA-100

If the solvent control value is within the normal range, a test material producing a positive response equal to twice the solvent control value for TA-98 and TA-100 is considered mutagenic.

(3) Pattern

Because TA-1535 and TA-100 are both derived from the same parental strain (G-46) and because TA-1538 and TA-98 are both derived from the same parental strain (D3052), to some extent there is a built-in redundancy in the microbial assay. In general, the two strains of a set respond to the same mutagen and such a pattern is sought. Generally, if a strain responds to a mutagen in nonactivation tests, it will do so in activation tests.

(4) Reproducibility

If a test material produces a response in a single test which cannot be reproduced in additional runs, the initial positive test data lose significance.

5. EVALUATION CRITERIA (Continued)

E. Evaluation Criteria for Toxicity

(1) Complete toxicity

When there are no revertants observed on the plate(s) treated with the test compound, the test compound is defined as toxic to all or any of the indicator strains at that (those) particular dose(s).

(2) Slight toxicity

When there are fifty or less percent revertants on the plate(s) treated with the test compound as compared to the solvent control plate(s), the test compound is defined as slightly toxic to all or any of the indicator strains at that (those) particular dose(s).

F. Relation Between Mutagenicity and Carcinogenicity

It must be emphasized that the Ames Salmonella/Microsome Plate Assay is not a definitive test for chemical carcinogens. It is recognized, however, that correlative and functional relations have been demonstrated between these two endpoints. The results of comparative tests on 300 chemicals by McCann et. al.¹ show an extremely good correlation between results of microbial mutagenesis tests and in vivo rodent carcinogenesis assays.

All evaluations and interpretation of the data to be presented in the final report will be based only on the demonstration, or lack, of mutagenic activity.

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Q.A. Inspection Statement
(reference 21 CFR 58.35(b)(7))

PROJECT 20988

LBI Assay No. 5394

TYPE of STUDY AMES PLATE TEST

This final study report was reviewed by the LBI Quality Assurance Unit on 12/5/80. A report of findings was submitted to the Study Director and to Management on 12/5/80.

The short-term nature of this study precluded inspection while it was in process. The Quality Assurance Unit inspects an in-process study of this type approximately once per month to assure that no significant problems exist that are likely to affect the integrity of this type of study.

Mitchell S. Edel

Auditor, Quality Assurance Unit

GENETICS ASSAY NO. 5394
LBI SAFETY NO. 6378

TOXIC EVALUATION OF
SAMPLE #1452
(WATER TANK DRAIN, END OF TEST)

IN THE
RODENT QUANTAL TOXICITY
ASSAY

FINAL REPORT

SUBMITTED TO:

ACUREX CORPORATION
485 CLYDE AVENUE
MOUNTAIN VIEW, CA 94042

SUBMITTED BY:

LITTON BIONETICS, INC.
5516 NICHOLSON LANE
KENSINGTON, MARYLAND 20795

LBI PROJECT NO. 22064

REPORT DATE: DECEMBER, 1980

PREFACE

This report contains a summary of the data compiled during the evaluation of the test compound. The report is organized to present the results in a concise and easily interpretable manner. The first part contains items I - VIII. Items I - III provide sponsor and compound identification information and identify the type of assay. The assay was conducted and evaluated according to procedures recommended in IERL-RTP Procedures Manual: Level 1 Environmental Assessment Biological Tests (Litton Bionetics, Inc., Kensington, MD, September, 1980, in press). Item IV provides the initiation and completion dates for the study, and Item V provides identification of supervisory personnel. Item VI identifies the tables and figures containing the data used by the study director in interpreting the test results. The interpretation itself is in Item VII. Item VIII provides the conclusion and evaluation.

The second part of the report, entitled Assay Design, describes the materials and procedures employed in conducting the assay. This part of the report also contains evaluation criteria used by the study director, and any appendices. The evaluation criteria are included to acquaint the sponsor with the methods used to develop and analyze the test results.

All test and control results presented in this report are supported by fully documented raw data which are permanently maintained in the files of the Department of Genetics and Cell Biology or in the archives of Litton Bionetics, Inc., 5516 Nicholson Lane, Kensington Maryland, 20795.

Copies of raw data will be supplied to the sponsor upon request.

- I. SPONSOR: Acurex Corporation
- II. MATERIAL (TEST COMPOUND): GENETICS ASSAY NO.: 5394
- A. Identification: Sample #1452 (Man Test 5/21/80, #5 Gal, water tank drain, end of test)
- B. Date Received: October 6, 1980
- C. Physical Description: Clear, colorless liquid
- III. TYPE OF ASSAY: Rodent Quantal Toxicity Assay
- IV. STUDY DATES:
- A. Initiation: October 22, 1980
- B. Completion: November 24, 1980
- V. SUPERVISORY PERSONNEL:
- A. Study Director: David J. Brusick, Ph.D.
- B. Laboratory Supervisor: Joan McGowan
- VI. RESULTS:
- The data are presented in Table 1 on pages 3 and 4.
- VII. INTERPRETATION OF RESULTS:
- The test material, Sample No. 1452, was concentrated 10-fold (as described under Assay Design) prior to administration by oral gavage to 10 male and 10 female weanling CD-1 mice. A single average dose of 5 ml/kg was given, which corresponded to 0.08 ml for the males and 0.07 ml for the females. The animals were observed for 14 days for toxic signs or lethality. Survivors were weighed and necropsied on Day 14 after dosing.
- All twenty animals survived the exposure with no evidence of any compound-related toxic signs. Both the male and female mice showed good weight gains, and no observations were obtained at necropsy that indicated compound-related lesions (Table 1). The test material was therefore considered to have no detectable toxicity in this assay, and according to the Evaluation Criteria, an LD50 determination was unnecessary.

VIII. CONCLUSIONS:

The test material, Sample No. 1452, concentrated 10-fold, had no detectable toxicity to weanling CD-1 mice in the Rodent Quantal Toxicity Assay. The applied dose was 5 ml/kg.

Submitted by:

Study Director

for: Brian C. Mynher
David J. Brusick, Ph.D.
Director
Department of Genetics
and Cell Biology

12/3/80
Date

TABLE 1

QUANTAL TOXICITY DATA WITH WEANLING MICE

Quantal Toxicity: Weanling mice
 Sponsor: Acurex Corporation
 Test Article: Sample No. 1452
 Vehicle: Not Applicable
 Study Dates: 11/10/80 to 11/24/80
 Animal Room No.: 4S
 Animals: Charles River CD-1 mice, P.O. 100760
 Dose: 5 ml/kg administered P.O.

Animal No.	Initial Weight gm	Final Weight gm	Visible Toxic Signs ^a	Gross Necropsy Findings
<u>Males</u>				
1101	16.1	24.2	NTS ^b	Small mucoid lump in bladder
1102	16.7	25.5	NTS	Both eyes opaque
1103	14.2	22.1	NTS	Small mucoid lump in bladder
1104	14.4	21.7	NTS	NSL ^c
1105	15.6	23.2	NTS	NSL
1106	13.5	22.2	NTS	Both eyes opaque
1107	16.2	23.7	NTS	NSL
1108	13.0	23.7	NTS	NSL
1109	14.8	23.8	NTS	•Both eyes opaque •Mesenteric lymph nodes slightly enlarged •Left kidney slightly pale
1110	16.6	25.2	NTS	Both eyes opaque

Mean Body Weight:

Initial 15.1 ± 1.3 gm (Standard Deviation)
 Final 23.5 ± 1.3 gm (Standard Deviation)

^a Animals observed for 14 days.

^b NTS = No Toxic Signs.

^c NSL = No Significant Lesions.

TABLE 1 (continued)
QUANTAL TOXICITY DATA WITH WEANLING MICE

Animal No.	Initial Weight gm	Final Weight gm	Visible Toxic Signs ^a	Gross Necropsy Findings
<u>Females</u>				
1111	14.1	19.8	NTS ^b	NSL ^c
1112	14.4	20.0	NTS	NSL
1113	14.3	20.4	Possible nasal irritation	Uterine horns enlarged/ fluid
1114	14.5	20.2	NTS	NSL
1115	12.5	19.0	NTS	NSL
1116	14.0	21.2	NTS	Uterine horns enlarged/ fluid
1117	13.3	18.7	NTS	NSL
1118	12.1	19.9	NTS	NSL
1119	13.5	19.7	NTS	Left kidney slightly smaller than right
1120	11.9	16.7	NTS	NSL

Mean Body Weight:

Initial 13.5 ± 1.0 gm (Standard Deviation)
Final 19.6 ± 1.2 gm (Standard Deviation)

^aAnimals observed for 14 days.

^bNTS = No Toxic Signs.

^cNSL = No Significant Lesions.

ASSAY DESIGN

RODENT QUANTAL TOXICITY ASSAY

This assay conforms to one of the bioassay tests in the US Environmental Protection Agency (EPA) IERL-RTP Procedures Manual: Level 1 Environmental Assessment Biological Tests (September, 1980). Level 1 bioassays obtain preliminary information on the harmful biological effects of chemicals found in industrial feed and waste streams.

1. OBJECTIVE

The objective of this assay was to evaluate the acute toxicity of a test material when administered by oral gavage to male and female weanling mice.

The assay consisted of recording any lethality and toxic signs that occurred over a 14-day period following a single treatment and then collecting necropsy information on animals that died or were killed at the end of the observation period.

2. TEST MATERIAL

A test material described as Sample #1452 (Man Test 5/21/80, #5 Gal, Water Tank Drain, End of Test) was received as a clear, aqueous liquid containing no obvious particulate material. The sample was stored at 4°C in its original one-gallon amber glass container.

An aliquot of 600 ml of test material was concentrated 10-fold to 60 ml by lyophilization prior to testing. A total volume of 1.5 ml of the concentrate was then utilized for oral dosing of a group of 20 weanling mice. The concentrate was stored at 4°C in an amber glass bottle.

3. EXPERIMENTAL DESIGN

Nine nursing female Charles River CD-1 mice with six pups each (three male and three female) were obtained from Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts on November 5, 1980. The birth date of the pups was October 19, 1980. The animals were quarantined for 5 days upon receipt. The litters were individually housed on Absorb-Dri bedding in polycarbonate cages and were cared for according to Litton Bionetics, Inc., Department of Genetics and LAMS Standard Operating Procedures. Purina certified laboratory chow and water (pH 2.5) were provided ad libitum. The pups were

3. EXPERIMENTAL DESIGN (continued)

maintained with mothers until weaned. The animals were identified by eartags and cage cards and were released for study on November 10, 1980.

Prior to initiation of dosing, ten male and ten female weanling mice were individually weighed; the volume of the test material to be administered was based on the mean weight values for each sex. The test material (10-fold concentrate of Acurex Sample #1452) was administered once at 5 ml/kg on November 10, 1980. The weanling mice were 23 days old. The animals were observed at the time of dosing, 2 hours later, and daily thereafter. On day 14, the surviving animals were fasted overnight. They were subjected to gross necropsy the next day.

4. ASSAY EVALUATION CRITERIA

Immediately following administration of the test material and at frequent intervals during the first day, observations of the frequency and severity of all toxic signs or pharmacological effects (as listed in Table A) will be recorded. Particular attention will be paid to the time of onset and disappearance of the signs. Daily observations will be made and recorded on all animals through a 14-day period. At termination of the observation period, all surviving animals will be weighed, killed, and then gross necropsies performed. Necropsies will also be performed on all animals that die during the course of this study.

If no mortality occurs in the quantal study, no further studies will be performed with the test substance and the LD₅₀ should be reported as greater than 5 ml/kg or 5 g/kg. The test material is ranked as having nondetectable toxicity (ND) at the maximum applicable dose (MAD). Effluent samples which produce harmful effects in vivo and do not result in deaths will be noted in the results summary. Such observations are difficult to quantitate but provide insight into the sublethal effects of a sample on rodents. Further investigations may be recommended from observations of nonlethal toxic effects.

If a single animal in the quantal study dies in the 14-day observation period, a quantitative study will be performed. An LD₅₀ will be calculated by the method of Litchfield and Wilcoxin. If the data are not suitable for calculation of a precise LD₅₀, i.e., total mortality occurs for the lowest dose, an estimate of the LD₅₀ could be made or the LD₅₀ could be expressed as 0.05 ml/kg or 0.05 g/kg or less. Occasionally, it may be necessary to use a different series of dosages in a repeat study to accurately locate the LD₅₀. The calculated LD₅₀ value is used to rank the toxicity of the test material according to the dose ranges presented in Table B.

TABLE A. DEFINITION OF PHARMACOLOGICAL TOXIC SIGNS

Organ System	Observation and Examination	Common Signs of Toxicity
CNS and somatomotor	Behavior	Change in attitude to observer, unusual vocalization, restlessness, sedation
	Movements	Twitch, tremor, ataxia, catatonia, paralysis, convulsion, forced movements
	Reactivity to various stimuli	Irritability, passivity, anaesthesia, hyperaesthesia
	Cerebral and spinal reflexes	Sluggishness, absence
Autonomic nervous system	Muscle tone	Rigidity, flaccidity
	Pupil size	Myosis, mydriasis
Respiratory	Secretion	Salivation, lacrimation
	Nostrils	Discharge
Cardiovascular	Character and rate of breathing	Bradypnoea, dyspnoea, Cheyne-Stokes breathing, Kussmaul breathing
	Palpation of cardiac region	Thrill, bradycardia, arrhythmia, stronger or weaker beat
	Events	Diarrhea, constipation, Flatulence, contraction
Gastrointestinal	abdominal shape	Unformed, black or clay colored
	feces consistency and color	
	Vulva, mammary glands	Swelling
Skin and fur	Penis	Prolapse
	Perianal region	Soiled
	Color, turgor, integrity	Reddening, flaccid skinfold, eruptions, piloerection
Mucous membranes	Conjunctiva, mouth	Discharge, congestion, hemorrhage cyanosis, jaundice
		Exophthalmus, nystagmus
Eye	Eyeball	Opacities
Others	Transparency	Subnormal, increased temperature
	Rectal or paw skin	Swelling
	Injection site	Abnormal posture, emaciation
	General Condition	

4. ASSAY EVALUATION CRITERIA (continued)

Observations are also made and recorded daily on all animals through the 14-day period. As in the quantal phase, no attempt is made to quantitate or rank the observations. The average animal body weight of each group is determined initially and at the termination of the experiment. The average weights and the weights as fractions of the control are reported for each dose level. Necropsy observations are recorded and reported.

TABLE B. ACUTE IN VIVO RODENT ASSAY EVALUATION CRITERIA

Toxicity ^a	Solids (LD ₅₀ in g/kg)	Liquids (LD ₅₀ in ml/kg)
High	<0.05	<0.05
Moderate	0.05 to 0.5	0.05 to 0.5
Low	0.5 to 5	0.5 to 5
Not Detectable	>5	>5

^aEvaluation criteria formulated by Litton Bionetics, Inc. for IERL-RTP Procedures Manual: Level 1 Environmental Assessment Biological Tests.

5. RECORDS TO BE MAINTAINED

All raw data, protocols, protocol modifications, test article weight and dispensation records and correspondence between LBI and the Sponsor are being maintained in a central file within the Department of Genetics and Cell Biology. These records will be filed under departmental assay number and held up to 2 years following submission of the final report to the Sponsor. After 2 years they will be transferred to the LBI archives for permanent storage.

Q.A. Inspection Statement
(reference 21 CFR 58.35(b)(7))

PROJECT 22064

LBI Assay No. 5394

TYPE of STUDY Rodent QUANTAL Toxicity Assay

This final study report was reviewed by the LBI Quality Assurance Unit on 12/10/80. A report of findings was submitted to the Study Director and to Management on 12/10/80.

The short-term nature of this study precluded inspection while it was in process. The Quality Assurance Unit inspects an in-process study of this type approximately once per month to assure that no significant problems exist that are likely to affect the integrity of this type of study.

Mitchell P. Ehrlich
Auditor, Quality Assurance Unit

SECTION 6
SAM IA WORK SHEETS

1. SOURCE/CONTROL OPTION: RESIDENTIAL fuernace M.P.N/HARDWICK LEVEL 1 X LEVEL 2 Page 1 of 5

2. EFFLUENT STREAM CD's STREAM 3. TOTAL MASS RATE OF DISCHARGE 98 g/s

CODE NO. NAME Q: g/s

4. COMPLETE THE FOLLOWING TABLE FOR THE EFFLUENT STREAM OF LINE 2

A	B	C	D	E	F	G	H	I	J	K	L	M
SPECIES OR MEO CATEGORY NAME	MEO ID NUMBER OR CATEGORY	SPECIES OR CATEGORY CONCENTRATION	HEALTH DMSO CONCENTRATION	MEO ASSUMED - HEALTH	ECOLOGICAL DMSO CONCENTRATION	MEO ASSUMED - ECOLOGY	DISCHARGE SEVERITY (ICD) - HEALTH	DISCHARGE SEVERITY (ICP) - ECOLOGICAL	HEALTH DMSO EXCEEDED	ECOLOGICAL DMSO EXCEEDED	HEALTH (BASED) (LINE 2)	ECOLOGICAL (BASED) (LINE 3)
UNITS												
ANIMATIC HYDROCARBONS	01	4g/m ³	4g/m ³	—			7.4x10 ⁻³				9.1	9.1
METHANE	01A020	2.6x10 ³	3.5x10 ⁵	01A020			1.2x10 ⁻³				7.25x10 ⁻¹	
ALDEHYDES	07A	1.0x10 ²	2.5x10 ²	07A020			4.0x10 ⁻¹				3.92x10 ⁻¹	
CARBOXYLIC ACIDS	08A0	2.0x10 ²	1.0x10 ³	08A020			2.0x10 ⁻¹				1.96x10 ⁻¹	
NAPHTHALENE	02A020	3.6x10 ⁻¹	2.2x10 ⁵	02A020			1.6x10 ⁻⁴				1.6x10 ⁻²	
PHENANTHRENE	01A180	2.0	1.6x10 ³	21A180			1.3x10 ⁻³				1.27x10 ⁻¹	
LITHIUM	27	2.4x10 ⁻¹	2.2x10 ⁻¹	27A020			1.1x10 ⁻²				1.1	

(IF MORE SPACE IS NEEDED USE A CONTINUATION SHEET)

5. TOTAL DISCHARGE SEVERITY HEALTH BASED (I col H) 5a 2.72x10⁻¹ ECOLOGICAL BASED (I col I) 5b 2.69x10⁻³

6. TOTAL WEIGHTED DISCHARGE SEVERITY HEALTH BASED (I col L) 6a 2.69x10⁻³ ECOLOGICAL BASED (I col M) 6b 9.1

(ENTER HERE AND AT LINE 8 OF THE SUMMARY SHEET)

Page 3 of 5

CONTINUATION SHEET FOR WORKSHEET

1. SOURCE/CONTROL OPTION RESIDENTIAL FURNACE MAN/HARDWIL

2. EFFLUENT STREAM CR3 STREAM

CODE NO. _____

A	B	C	D	E	F	G	H	I	J	K	L	M
SPECIES OR NEG. CATEGORY NAME	NEG. ID NUMBER OR CATEGORY	SPECIES OR CATEGORY CONCENTRATION	HEALTH BASED CONCENTRATION	NEG. ASSUMED HEALTH	ECOLOGICAL DMS CONCENTRATION	NEG. ASSUMED ECOLOGY	DISCHARGE SEVERITY (C.D.)	DISCHARGE - ECOLOGICAL (C.F.)	HEALTH BASED DMS	ECOLOGICAL DMS	HEALTH BASED P.L.M. 2	ECOLOGICAL BASED P.L.M. 2
UNITS												
CO	43800	2.1×10^{-4}	4×10^{-4}	43800			7.7×10^{-1}				7.6×10^1	
SILICON	43	27.9×10^1	1.0×10^{-4}	43800			27.9×10^{-3}				27.7×10^{-1}	
GERMANIUM	44	5.3×10^{-2}	5.6×10^{-2}	44800			5.9×10^{-5}				5.8×10^{-3}	
TIN	45	1.1×10^{-1}	1.0×10^{-4}	45800			1.1×10^{-5}				1.1×10^{-3}	
LEAD	46	2.8	1.5×10^2	46800			1.9×10^{-2}				1.9	
NO.	47000	9.7×10^4	9×10^3	47800			1.1×10^1		✓		1.1×10^3	
PHOSPHORUS	48	2.3	1.0×10^2	48800			2.2×10^{-2}				2.2	
ARSENIC	49	1.7×10^{-1}	2.0	49800			8.5×10^{-2}				8.4	
ANTIMONY	50	5.1×10^{-2}	5×10^{-1}	50800			1.0×10^{-4}				9.8×10^{-3}	
SULFUR	53	4.8×10^2	1.0×10^3	53800			4.8×10^{-1}				4.7×10^1	
SO2	53800	1.3×10^5	1.3×10^4	53800			1.0×10^1		✓		9.8×10^2	

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CONTINUATION SHEET FOR WORKSHEET

1. SOURCE/CONTROL OPTION RESIDENTIAL FURNACE MAIN/HARDWICK

2. EFFLUENT STREAM GAS STREAM

CODE NO. _____ NAME _____

LEVEL 1 2

LEVEL 2 _____

A	B	C	D	E	F	G	H	I	J	K	L	M	N
SPECIES OR WED CATEGORY NAME	WED NUMBER OR CATEGORY	WEDS OR CATEGORY CONCENTRATION	HEALTHY WED CONCENTRATION	WED ASSUMED - HEALTHY	ECOLOGICAL WED CONCENTRATION	WED ASSUMED - ECOLOGY	DISCHARGE - HEALTHY	DISCHARGE - ECOLOGY	DISCHARGE - HEALTHY	DISCHARGE - ECOLOGY	WEDS OR CATEGORY	WEDS OR CATEGORY	WEDS OR CATEGORY
UNITS		$\mu\text{g}/\text{m}^3$	$\mu\text{g}/\text{m}^3$										
SELENIUM	54	2.5×10^{-1}	2.0×10^{-2}	50000			1.3×10^{-3}						
TELLURIUM	55	4.2×10^{-1}	1.0×10^{-2}	55000			4.2×10^{-3}						
FLUORINE	56	1.4×10^{-1}	2.0×10^{-3}	56000			7.0×10^{-3}						
BROMINE	58	1.7	1.0×10^{-4}	58000			1.7×10^{-4}						
YTRIUM	61	2.8×10^{-1}	1.0×10^{-3}	61000			2.8×10^{-4}						
TITANIUM	62	1.3×10^{-1}	6.0×10^{-3}	62000			2.2×10^{-3}						
Vanadium	65	1.1	5.0×10^{-2}	65000			2.2×10^{-3}						
TANTALUM	67	5.1×10^{-1}	5.0×10^{-3}	67000			1.0×10^{-4}						
Chromium	68	3.4	1.0	68000			3.4						
MOLYBDENUM	69	2.8	5.0×10^{-3}	69000			5.6×10^{-4}						
MANGANESE	71	1.4	5.0×10^{-3}	71000			2.8×10^{-4}						

Page 5 of 5

CONTINUATION SHEET FOR WORKSHEET

1. SOURCE/CONTROL OPTION RESIDENTIAL FURNACE M.A.N./HARDWICK

2. EFFLUENT STREAM GAS STREAM

LEVEL 1 2

LEVEL 2 2

SPECIES OR NO. CATEGORY NAME	USE & NUMBER OF CATEGORY	C	D	E	F	G	H	I	J	K	RECALCULATED CONCENTRATION		M
											RECALCULATED CONCENTRATION	RECALCULATED CONCENTRATION	
IRON	72	1.5x10 ⁻¹	1.0x10 ⁻³	72000			1.5x10 ⁻²					1.47	
COPPER	74	1.1x10 ⁻¹	5.0x10 ⁻¹	74000			2.2x10 ⁻³					2.2x10 ⁻¹	
NICKEL	76	8.6	1.5x10 ⁻¹	76000			5.7x10 ⁻¹					5.6x10 ⁻¹	
COPPER	78	5.9	2.0x10 ⁻²	78000			3.0x10 ⁻²					3.0	
SILVER	79	5.4x10 ⁻³	1.0x10 ⁻¹	79000			5.4x10 ⁻⁴					5.3x10 ⁻²	
ZINC	81	7.5	4.0x10 ⁻³	81000			1.9x10 ⁻³					1.9x10 ⁻¹	
MERCURY	83	4.8x10 ⁻¹	5.0x10 ⁻¹	83000			9.6x10 ⁻³					9.4x10 ⁻¹	
ANTHRACENE	84	1.1x10 ⁻¹	1.1x10 ⁻⁵	84000			1.0x10 ⁻⁶					9.8x10 ⁻⁵	
PERILUM	84	1.5x10 ⁻¹	3.7x10 ⁻⁴	84000			4.1x10 ⁻⁶					4.0x10 ⁻⁴	
NEODYMIUM	84	5.4x10 ⁻³		84000									

1. SOURCE/CONTROL OPTION: RESIDENTIAL FURNACE P.A.N. HADWICK LEVEL 1 X Page 1 of 4
LEVEL 2 ---

2. EFFLUENT STREAM: WASTE WATER (LIQUID STREAM) CODE NO. --- 3. TOTAL MASS RATE OF DISCHARGE: 4.7x10⁻² g/s

4. COMPLETE THE FOLLOWING TABLE FOR THE EFFLUENT STREAM OF LINE 2

A	B	C	D	E	F	G	H	I	J	K	L	M
SPECIES OR NO.	HEALTH OR CATEGORY	SPECIES OR CATEGORY	HEALTH OR CATEGORY	HEALTH OR CATEGORY	HEALTH OR CATEGORY	HEALTH OR CATEGORY	DISCHARGE SEVERITY (I COI M) 5a	DISCHARGE SEVERITY (I COI M) 5b	HEALTH OR CATEGORY	HEALTH OR CATEGORY	HEALTH OR CATEGORY	HEALTH OR CATEGORY
UNITS												
NAPHTHALENE	21A020	4.0x10 ⁻¹	7.5x10 ⁵	21A020	1.0x10 ²	21A020	5.3x10 ⁻⁷	4.0x10 ⁻³	---	---	---	---
PHENANTHRENE	21A180	8.0x10 ⁻²	2.4x10 ⁴	21A180	---	---	3.3x10 ⁻⁶	---	---	---	---	---
MAGNESIUM	33	1.0x10 ²	9.0x10 ⁴	33A000	8.7x10 ⁴	33A000	1.1x10 ⁻³	1.2x10 ⁻³	---	---	---	---
CALCIUM	34	2.0x10 ⁴	2.4x10 ⁵	34A000	1.6x10 ⁴	34A000	4.2x10 ⁻²	6.2x10 ⁻¹	---	---	---	---
STRONTIUM	35	2.0x10 ¹	4.6x10 ⁴	35A000	---	---	4.4x10 ⁻⁴	---	---	---	---	---
Barium	44	2.0	8.4x10 ³	44A000	---	---	2.4x10 ⁻⁴	---	---	---	---	---
LEAD	46	7.0x10 ¹	2.5x10 ²	46A000	5.0x10 ¹	46A000	2.8x10 ⁻¹	1.4	✓	✓	✓	6.6x10 ⁻²

5. TOTAL DISCHARGE SEVERITY: HEALTH BASED (I COI M) 5a 1.85x10⁻² ECOLOGICAL BASED (I COI M) 5b 1.25x10⁻⁴
(ENTER HERE AND AT LINE 8 OF THE SUMMARY SHEET)

6. TOTAL WEIGHTED DISCHARGE SEVERITY: HEALTH BASED (I COI M) 5a 8.7 ECOLOGICAL BASED (I COI M) 5b 5.8x10⁻²
(ENTER HERE AND AT LINE 8 OF THE SUMMARY SHEET)

Page 2 of 4

CONTINUATION SHEET FOR WORKSHEET

1. SOURCE/CONTROL OPTION RESIDENTIAL WASTEWATER M.A.N. HARDWICK

2. EFFLUENT STREAM WASTEWATER (LIQUID STREAM)

CODE NO. _____ NAME _____

A	B	C	D	E	F	G	H	I	J	K	L	M
SYMBOL OR NAME	UNIT	CONCENTRATION	CONCENTRATION	CONCENTRATION	CONCENTRATION	CONCENTRATION	CONCENTRATION	CONCENTRATION	CONCENTRATION	CONCENTRATION	CONCENTRATION	CONCENTRATION
NITRATE	54	49.14	49.14	49.14	49.14	49.14	49.14	49.14	49.14	49.14	49.14	49.14
SULFATE	55	1.0x10 ²	1.0x10 ²	1.0x10 ²	1.0x10 ²	1.0x10 ²	1.0x10 ²	1.0x10 ²	1.0x10 ²	1.0x10 ²	1.0x10 ²	1.0x10 ²
SELENIUM	56	1.0x10 ²	1.0x10 ²	1.0x10 ²	1.0x10 ²	1.0x10 ²	1.0x10 ²	1.0x10 ²	1.0x10 ²	1.0x10 ²	1.0x10 ²	1.0x10 ²
TELLURIUM	57	2.0x10 ¹	2.0x10 ¹	2.0x10 ¹	2.0x10 ¹	2.0x10 ¹	2.0x10 ¹	2.0x10 ¹	2.0x10 ¹	2.0x10 ¹	2.0x10 ¹	2.0x10 ¹
FLUORINE	58	1.0x10 ²	1.0x10 ²	1.0x10 ²	1.0x10 ²	1.0x10 ²	1.0x10 ²	1.0x10 ²	1.0x10 ²	1.0x10 ²	1.0x10 ²	1.0x10 ²
CHLORIDE	59	1.0x10 ³	1.0x10 ³	1.0x10 ³	1.0x10 ³	1.0x10 ³	1.0x10 ³	1.0x10 ³	1.0x10 ³	1.0x10 ³	1.0x10 ³	1.0x10 ³
BROMINE	60	1.0x10 ²	1.0x10 ²	1.0x10 ²	1.0x10 ²	1.0x10 ²	1.0x10 ²	1.0x10 ²	1.0x10 ²	1.0x10 ²	1.0x10 ²	1.0x10 ²
SCANDIUM	61	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
YTRIUM	62	5.0x10 ¹	5.0x10 ¹	5.0x10 ¹	5.0x10 ¹	5.0x10 ¹	5.0x10 ¹	5.0x10 ¹	5.0x10 ¹	5.0x10 ¹	5.0x10 ¹	5.0x10 ¹
TITANIUM	63	2.0x10 ²	2.0x10 ²	2.0x10 ²	2.0x10 ²	2.0x10 ²	2.0x10 ²	2.0x10 ²	2.0x10 ²	2.0x10 ²	2.0x10 ²	2.0x10 ²
ZINC	64	3.0x10 ¹	3.0x10 ¹	3.0x10 ¹	3.0x10 ¹	3.0x10 ¹	3.0x10 ¹	3.0x10 ¹	3.0x10 ¹	3.0x10 ¹	3.0x10 ¹	3.0x10 ¹

LEVEL 1

1. SOURCE/CONTROL OPTION RESIDENTIAL FURNACE M.A.N. HADWICK
EFFLUENT STREAM

WASTE WATER (LIQUID STREAM)

CODE NO.

ITEM

[illegible]